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ATLAS AND PRINCIPLES
OF
BACTERIOLOGY
AND TEXT-BOOK OF
SPECIAL BACTERIOLOGIC DIAGNOSIS

BY

PROF. DR. K. B. LEHMANN
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AUTHORIZED TRANSLATION FROM THE SECOND ENLARGED AND REVISED GERMAN EDITION

EDITED BY
GEORGE H. WEAVER, M.D.
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PART II—TEXT

PHILADELPHIA AND LONDON
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1901

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EDITOR'S PREFACE.

THE scope and purpose of this work are sufficiently stated in the authors' preface. The need of such a work has often been felt in directing the work of advanced students especially, and it is with the hope of aiding them that it has been undertaken to place the contents of this work within their easy reach. Because of numerous mistakes in the references in the original, all of those which refer to Plates in the atlas have been verified or corrected, and also as many of those which refer to the literature as were accessible. A few references to original articles in English have been inserted.

37451

FROM THE
PREFACE TO THE FIRST EDITION.

Doubt, honestly arrived at and acknowledged, is better than apparent certainty without a statement of those things upon which it depends.

FOR years my brother, J. F. Lehmann, the publisher in Munich, has requested me to furnish him for his "Medical Atlases" one which would simplify bacteriologic diagnosis. After I had long refused to undertake the vast labor which this would necessitate, a fortunate circumstance in the summer of 1894 led me to accept the plan. I discovered in Dr. R. Neumann, who was working in bacteriology in my institute, so excellent a talent for drawing and painting that I proposed to him that he undertake the work with me. Whether we have solved the problem remains for the critics to decide. It seems to me that the plates, painted by Dr. Neumann with untiring zeal under my continual supervision, and carefully reproduced by the lithographer Fr. Reichhold in Munich, are a useful addition to our means of teaching. With few exceptions, the reproduction leaves little to be desired. At least, we have had the satisfaction of finding the pictures of great advantage in our own work and in that of numerous gentlemen working in our institute. We carried out many investigations regarding the method of illustrating before selecting the one employed, which may be considered as almost entirely satisfactory.

At the present time, when, properly, photography is so much used for the objective representation of objects in the natural sciences, especially those of bacteriology, many will look with suspicion upon a painted bacteriologic atlas. We hope, however, that the unprejudiced critic will concede that for certain objects (stab, streak, and potato cultures) a well-colored representation surpasses the best photograph, and that for a second group of pictures (plate-colonies slightly magnified) a drawing, which can alone do justice to the depth of the object, is at least equal to a photograph. We gladly acknowledge that for the representation of individuals magnified 1000 times photography is the best method ; but there is now scarcely any doubt that for the practical differential diagnosis of bacteria, only in somewhat rare cases is the picture of the individual of primary importance. We have, moreover, sought to take advantage of the photographic method when the individuals were to be represented, by comparing the splendid photographs in the atlas of C. Fränkel and R. Pfeiffer, and also those in the literature (by Löffler, Heim, Roux, etc.), with our own preparations.

The choice of varieties for illustration was often very difficult. To our great pleasure, we were able to present, with the exception of about 4 per cent., only originals in the atlas ; while, naturally, those required as supplements to the text are more often copies. In the latter case the original source is always given. Varieties important from a medical standpoint, especially when they present any visible characteristics, could scarcely be omitted; also, almost all varieties pathogenic for animals are introduced. Chromogenic, zymogenic, and saprogenic bacteria were never, to our knowledge, so extensively represented before; nevertheless, in this portion a careful choice was required. We acknowledge that some among those selected might have been omitted, and others chosen.

The text is divided into a general part, which I have prepared alone, and a special part, in which I have received the constant cooperation of Dr. Neumann.

The general part furnishes a condensed survey of the principal properties of bacteria so far as they are of practical value, especially so far as they are of diagnostic aid. It is assumed that the reader has mastered the ordinary elements of bacteriologic technic, but at the request of the publisher we have appended a short list of media rules for stains, etc., and constant reference is made to them. More complete information in these matters is furnished by the well-known works of C. Fränkel, Günther, Hüppe, and in especially painstaking minuteness by the exhaustive work of Heim: "Lehrbuch der bacteriologischen Untersuchung und Diagnostik."

The special part attempts to give, so far as possible in a natural botanical arrangement, a complete description of the important varieties, with constant reference to less important ones which for any reason are worthy of notice. Those which we have described in detail we have also thoroughly investigated, thus supplying many previous omissions.[1] A great part of the related species have been studied so far as time, strength, and opportunity allowed.

Of new "species," we have introduced only a very few; identical varieties described under various names we have grouped together; and in many places we have directly tried to build up a natural system. It was evidently impossible to offer anything complete or final in the treatment of the non-pathogenic varieties.

Moreover, we are of the opinion that the advance of bacteriology, which we seek, especially the elucidation of the questions of variability, relation, distribution in and outside of living organisms, etc., cannot be accomplished by one or several, but only by systematic national—or, better, international—cooperation of investigators under a grand division of labor. One task for this cooperation would be to so improve and remodel the present often unprecedentedly arbitrary and unscientific nomenclature of fission-fungi that it will not challenge the derision of every scientist. (Compare Introduction to Special Part.)

Not infrequently our observations did not agree with certain statements of various respected observers, but we have always expressly acknowledged the same, and especially have pointed out the contradictions and defects, hoping thus to do service.

For an extensive reference to literature we have found no room, but have only employed citations to facilitate detailed studies, especially pointing out recent reviews with numerous references. Every bacteriologic investigator will be unable to dispense with the aids which we have employed: Centralblatt für Bakteriologie und Parasitenkunde (Redakteur Uhlworm, Kassel, seit 1887), Baumgarten's Jahresbericht über die pathogenen, und Koch's Jahresbericht über die zymogenen, etc., Organismen. By their comprehensive index they quickly furnish a complete abstract of literature.

---

[1] If this were conscientiously done by all editors of bacteriologic works, there would be at least a partial elimination of the varieties which are non-critically enumerated, absolutely insufficiently described, and often repeated under different names.

If we have been able to somewhat further the diagnosis of bacteria, to lighten the task of the beginner, to indicate the numerous difficulties of this work, which are partly undetermined and too little appreciated, then we are rewarded for the great labor which we have expended. We hope especially to furnish the student in bacteriology a substantial aid, and to make it possible for him to better appreciate what is seen and heard. We beg our critics not to censure too strongly defects and mistakes, which necessarily entered because of the enormous material.

PROF. DR. K. B. LEHMANN.

PREFACE TO THE SECOND EDITION.

SOONER than we dared to hope, a large German edition of this work has been exhausted; the English, Italian, and Russian editions also have found a large sale. We accept this as an indication of the practical value of the book. With special pleasure we have observed in the numerous reviews of the book that its reformative tendency in regard to the grouping of varieties of bacteria, the strict division of the system especially, the rational naming of bacteria, etc., have found warm praise. The text-books of Heim and Mez have accepted our nomenclature entirely or in part. For many new names in Flügge-Kruse's work, which appeared a few months after ours, according to the rule of botanical systems, the priority remains with us. Moreover, where we have found that properly selected names, older than those which we chose in the first edition, existed, we have naturally strictly adhered to the rule of priority. We affirm with pleasure that, because of our exact observations and of reliable statements in the literature, the carefully championed view of the exceedingly great variability of bacteria finds more and more recognition, and that the authors who to-day describe "new species" are in the main fewer, as is witnessed by the intelligent views advanced by the collection of bacteriologists in New York in 1895 (C. B. xx, 450).

The opinion advanced from an esteemed source, that the constant emphasis of variability, of the limits of our knowledge, and of the uncertainty of known methods, may sometimes discourage the beginner, may not be entirely unfounded. Yet we believe this absolute frankness to be an advantage, even if thereby the dogmatic sharpness of the statements should sometimes suffer. With beginners one may and must leave much unsaid in order not to confuse; but ever so short a text-book can only claim the designation of science if the student can follow the author's thoughts. Besides, for the learner there is no greater satisfaction, when he comes upon difficulties, than the certain statement that, in a certain point, the imperfection of our knowledge, and not his inability, is the cause of the difficulty.

The fruitful labors of all investigators in the field of bacteriology made necessary a complete revision of the text in both the general and special parts. In the general part the discussion upon the causes of disease, disposition, and immunity is substantially extended. Beginning with page 119 is an exposition of the most important botanical points of view which are important in classifying and properly naming fission-fungi. In the special part, in fifty varieties dependent upon autopsies, we have made additions and improvements; about eighty varieties are newly introduced. We have especially undertaken fundamentally new work upon the causes of diphtheria and tuberculosis, together with the related varieties. It is hoped that the value of the atlas is essentially increased by the introduction of nine new plates, which replace three old ones (diphtheria and the allied bacteria, varieties related to the tubercle bacillus, gonorrhea, and pest).

The literature of the past three years has been conscientiously studied; many statements are substantiated, and everything which seemed of importance in the publications up to about June, 1899, is taken up. We hope that we have made a proper selection from the almost immeasurable material, which increases daily. Perfection, naturally, we cannot expect: some small mistakes and oversights could not be avoided. The division of the work was the same as in the first edition.

K. B. Lehmann.
R. O. Neumann.

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PART I.

GENERAL BACTERIOLOGY.

EXPLANATION OF ABBREVIATIONS EMPLOYED IN REFERENCES.

A. H. = Archiv für Hygiene. München. Oldenbourg since 1883.
A. G. A. = Arbeiten aus dem Kaiserlichen Gesundheitsamt. Berlin. Springer since 1885.
A. K. = Arbeiten aus dem bakteriologischen Institut der techn. Hochschule zu Karlsruhe. Edited by Prof. Dr. L. Klein and Prof. W. Migula since 1894.
A. P. = Annales de l'Institut Pasteur. Paris. Masson since 1887.
C. B. = Centralblatt für Bakteriologie und Parasitenkunde. Jena. Fischer. Since 1894 it has been divided into two parts.
C. B. L. = Centralblatt für die landwirtschaftlichen, phytopathologischen und zymotechnischen Anwendungen der Mikrobiologie.
H. R. = Hygienische Rundschau. Berlin. Since 1890.
Z. H. = Zeitschrift für Hygiene. Leipzig. Veit since 1886.
Flügge = Flügge: Die Mikroorganismen. Third edition. Leipzig, 1896.
Heim = Heim: Lehrbuch der Bakteriologie. Second edition. Stuttgart, 1890.
Kitt = Kitt: Bakterienkunde für Tierärzte. Third edition. Wien, 1896.
Zimmermann I and II = O. E. R. Zimmermann: Die Bacterien unserer Trink- und Nutzwässer. Chemnitz, I, 1890; II, 1894.
Migula, Schiz. = Migula, Schizophyta. Separate reprint from "Die natürl. Pflanzenfamilien von Engler und Prantl." Leipzig, 1896.
Migula, Sys. = Migula, System der Bakterien. Volume I, General Part. Jena, 1897.
Eisenberg = Bakteriologische Diagnostik von James Eisenberg. Hamburg and Leipzig, 1891. Third edition.
Lafar = Lafar: Technische Mykologie. Volume I. Schizomycetengärungen. Jena, 1897.
Günther = Einführung in das Studium der Bakteriologie. Fifth edition. Leipzig, 1898.
Zopf = Die Spaltpilze. Breslau. Third edition.

The references to illustrations in the atlas are given thus: the Plates with Arabic, the Figures with Latin numerals. Thus, 5, VIII signifies Plate 5, Figure VIII.

BACTERIOLOGY.

A. Introduction to the Morphology of Bacteria.

By **bacteria** (**spaltpilzen, schizomycetes** of Nägeli) we understand a very large group of lower vegetable organisms, morphologically very simple and uniform, but biologically extraordinarily differentiated, which are so connected with both the lower algæ [1] and fungi by transition forms that a sharp separation by an accurate definition is difficult. Arthur Meyer emphasizes the relationship of the spore-forming varieties to the ascomycetes, in which the spore-forming cells appear as asci. Indeed, bacteria bear a great resemblance to the simple flagellata, which are usually conceived as animals. [2]

The following definition may at least serve the practical requirements of experimental bacteriology.

Small unbranched [3] *cells, rarely more than 2, hardly ever 3–5 μ in thickness, almost* [4] *always without chlorophyl, spher-*

---

[1] Recently we have learned that the green lower algæ also possess parallel colorless forms, which can be obtained from them by cultures (Beyerinck); compare also Ludwig, C. B. L. II, 348.

[2] Compare Bütschli in Bronn's Klassen des Tierreiches, Bd. I, Abt. II, Mastigophora.

[3] Regarding the branching forms nearly related to bacteria compare p. 19.

[4] Practically, important bacteria with chlorophyl are unknown. Yet the green tadpole bacillus (Kaulquappenbacillus) of J. Frenzel must be recognized as a bacterium (Z. H. XI, 207). There is more doubt as to the relation of Dangeard's Eubacillus multisporus to the bacteria (C. B. X, 745). L. Klein described colorless varieties with bluish-green spores (C. B. VII, 440).

ical, rod, thread, or spiral in form, with no organs except flagella which are used for locomotion. Vegetative increase is by transverse, very rarely by longitudinal division. A series of varieties form roundish, endogenous resting spores; in others there have been, or asserted to have been, observed conidia-like formations (arthrospores). Other means of propagation have not been observed.

Bacteria occur, so far as we know, only in the following forms, which were first perfectly named by H. Buchner:

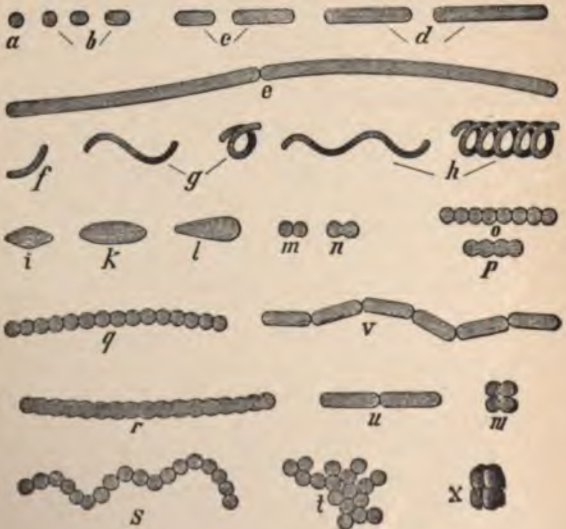

Fig. 1.—Forms of bacteria (after Buchner).

Individual Growth Forms.

Spherical form (Kugelform) (*a*).
Oval form (Ovalform) (*b*): length, at most 2 × the width.
Short rods (Kurzstäbchen) (*c*): length, 2–4 × width.
Long rods (Langstäbchen) (*d*): length, 4–8 × width.
Thread forms (Fadenform) (*e*).
Half screw (Halbschraube) = comma (*f*): a very short segment of a screw; at most, half the turn of a screw.
Short screw (Kurzschraube) (*g*): a short turn of a screw.

Long screw (Langschraube) = spiral form (*h*): all screw forms possess either steep or flat turns.

Spindle forms (Spindelform) (*i*).

Oval rods (Ovalstäbchen) (*k*) are differentiated from the spindle form by less tapering ends, from the oval form by their greater length = 2–4 × the width.

Clubbed form (Keulenform) (*l*).

Growth Groupings.

Diplococcus (Doppelkugel) (*m*) with barely perceptible separation: Biscuit form (*n*).

Streptococcus brevis (Kugelreihe) (*o*) up to 8 cocci; with barely perceptible separation: Torula form (*p*).

Streptococcus longus (Kugelfaden)(*q*), or, if bent, Rosary form (Rosenkranzform) (*s*); with barely perceptible separation: Torula threads (*r*).

Staphylococcus (Traubenform) (*t*). Diplobacillus (Doppelstäbchen) (*u*). Jointed threads (giederfaden) (*v*).

Tetrad (Tetradenform) (*w*): plane grouping of 4, 8, 16, etc., cells.

Sarcina (Würfelform) (*x*), cubical form: solid grouping of 8, 32, etc., cells.

Branching, *i. e.*, springing up of a side bud, was until recently unknown in connection with bacteria, and it is, at all events, rare. Besides in others, it is well established in the so-called tubercle and diphtheria bacilli as a frequent appearance, and thus it is demonstrated that here forms occur which do not strictly belong to bacteria.

Exceptionally, true branching appears to occur in other varieties. Heim mentions it in Bact. fluorescens. Vincenzi (C. B. XIV, 149) appears to have observed the same in tetanus, but in spite of special care, we have made no similar observations.

Often **pseudodichotomy** is confused with branching and dichotomy. According to Babès (Z. H. XX, 412), it occurs not infrequently in the most typical bacteria, and consists in this, that either the lower member of a thread grows past the side of the upper member, or that, in a row of cocci, the division of a coccus parallel to the direction of the string suddenly creates the beginning of a second thread. Stolz (C. B. XXIV, 337) has recently studied exhaustively

---

[1] Some authors falsely designate this true branching as true dichotomy, but true **dichotomy** means, according to botanical usage, only the division of the growing ends of threads into two equal twigs, and it is not certainly known to occur in bacteria.

and represented this abnormal division in streptococci. It occurs very frequently; indeed, we have often seen it.

Regarding the **structure of the bacterial cell,** much has been recently written. I must limit myself to what seems to me the most probable.

According to Alfred Fischer,[1] the conditions are very simple (Fig. 3): The bacteria consist of a cell-membrane, a protoplasmic layer, and a **central fluid.** Regarding a **nucleus** see below. In saline solutions (sodium chlorid, potassium nitrate, etc.) there occurs, the more concentrated the solution the more rapidly, through abstraction of water, a "**plasmolysis,**"—*i. e.*, a contraction of the mass of protoplasm with partial separation from the cell-membrane.[2] Thus are explained many clear vacuoles which occur in an ordinary cover-glass preparation of many bacteria (for example, B. typhi), and which were formerly

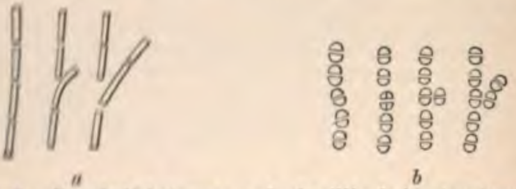

Fig. 2.—Pseudodichotomy: *a*, In bacilli; *b*, in streptococci.

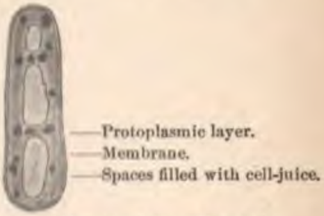

Fig. 3.—Bacillus oxalaticus Migula (after Migula).

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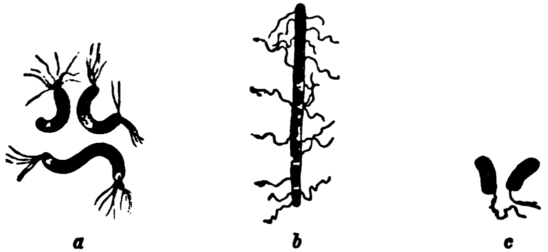
[1] Untersuchungen über Bakterien, 1894. Berlin. Separatabdruck aus den Jahrbüchern für wissenschaftl. Botanik, XXVII, Heft 1; and Untersuchungen über den Bau der Cyanophyceen und Bakterien., Jena, 1897.

[2] Frequently the drying on the cover-glass is sufficient to produce a picture of plasmolysis.

looked upon as spores. (Compare Fig. 4, *a*, *b*, *c*.) In water, this shrinking rapidly disappears, and also under the prolonged action of saline solution.

Migula (A. K., Bd. I), simultaneously and independently, reaches the same opinion as A. Fischer regarding the very large *Bacillus oxalaticus*, a spore-forming variety related to the hay bacillus. It happens especially in this that in the pressing outward of the protoplasmic layer, the central fluid space becomes distinct; in dehydrating media it becomes *smaller;* in water, *larger.*

While the botanists have hitherto sought in vain for a true **nucleus,** Arthur Meyer would recognize it in small, single, oval granules, staining with Ruthenium red and potassium iodid (Flora, 1897, Band 84, 185). Compare also the hitherto scarcely studied observations of A. Wagner (C. B. XXIII, 433), according to which nuclei were easily stained by primulin and hot Bordeaux red.



a, Spirillum undula. *b*, Bacillus Solmsii. *c*, Vibrio choleræ.
Fig. 4.—Plasmolysis (after A. Fischer).

In the interior of bacterial cells there are found, after proper staining, very many varieties of peculiar **granules,** which Babès, who discovered them, named **metachromatic bodies** (*i. e.*, staining differently from the cell-body). Ernst, the first accurate investigator of these bodies, called them **nuclei** or **sporogenic granules.**

While I refer to the literature of Babès (Z. H. XX, 412), which is rich in controversy, I give only the seductively clear view of one of the latest investigators of the subject, R. Bunge. Bunge (Fort. der Med., XIII, 813 and 853) distinguishes:

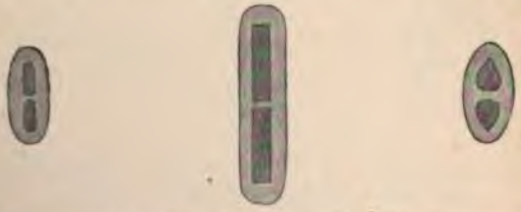
1. *Ernst's granules.* They stain blackish-blue when treated with warm Löffler's methylene-blue, washed in water, and after-stained with Bismarck brown. These granules are entirely absent in many

spore-forming varieties (anthrax, megatherium), and in others it can be shown that they have nothing to do with spores. They are therefore granules of unknown rank.

2. *Antecedents of spores* (*Bunge's granules*). Small granules, usually occurring multiple in sporulating cells, not staining according to Ernst's method, but, on the contrary, in *boiling* Löffler's solution. They are best shown, after previous treatment of the dried preparation with chromic acid, sodium peroxid, or hydrogen peroxid, according to the ordinary spore staining. (See Technical Appendix.) The perfected spores are formed by the union of many small antecedents.

Bunge explains the controversy which has occurred as due to much confusion regarding the two varieties of granules.

Regarding the **cell-membrane,** it is especially to be remarked that it often appears somewhat swollen, and not sharply outlined from without. In many bacteria (**"capsule-bacteria"** of authors) the thickening of the membrane or its outer layer is so extreme that the bacteria appear surrounded by a true **mucous envelope** or **capsule,** which is distinguished by its *slight* staining property with anilin dyes. It is an interesting fact that most of these capsule-forming bacteria only form these envelopes if growing either in the animal body or upon very special media,—fluid blood-serum, bronchial mucus, and also, according to Paulsen, upon milk.[1] Upon gelatin, agar, and potato, nothing



Bacterium pneumoniæ (Friedländer). Bacillus anthracis (Cohn). Streptococcus lanceolatus (Gamal.).

Fig. 5.—Capsule-formation (schematic).

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[1] Whether exquisite capsule-formation always occurs on these nutrient media, appears undecided. Recently, also, various authors have pointed out that **capsule-like formations** can be demonstrated in a **wider field** in the bacterial kingdom. Johne has described a method (see Technical Appendix) for anthrax by which the capsule can be

of these capsules appears. See also, in the special part, Streptococcus involutus and mesenterioides. Extensive review of literature by Binaghi, C. B. L. IV, 919.

Characteristic unilateral thickenings or swellings of the bacterial membrane are presented by **Bact. pediculatum**, which is described as an uncommon cause of the "frog-spawn disease" of sugar factories (Fig. 6).

Regarding especially striking membrane thickening at the ends of **threads (club formation)**, see those of actinomycetes.

The outer surface of bacteria is often perfectly smooth and without appendages in the short bacilli, and almost always in the spherical forms, but the longer rods and spiral forms are usually provided with delicate single or multiple **flagella.** These are often distributed over the whole body of the bacterium, often form only a little bunch at one end, and often there is found but a single polar flagellum. Bacteria with polar flagella, shortly before division, show a single flagellum or a bunch of flagella at *each* end. As A. Fischer particularly showed, flagella are not of the nature of retractile and extending pseudopodia, but true hair-like outgrowths. For the demonstration of flagella it is necessary to treat the bacteria with especially powerful staining agents. In this process the capsules of the bacteria, which remain unstained in the ordinary method, are stained, and so the bacteria appear very much thickened. Occasionally wide layers of capsule remain unstained and the flagella are then set upon a narrow ring-like halo, separated from the bacillus by a colorless zone (Zettnow, von Stöcklin, A. Fischer). Unfortunately, very many of the procedures

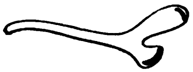

Fig. 6.—Bact. pediculatum (after Koch and Hosäus).

made plainly visible; also in this manner distinct capsules are obtained in B. megatherium, oxalaticus, etc. Babès has depicted capsules in connection with the Streptococcus pyogenes, and we have ourselves occasionally seen similar formations in the case of many bacteria.

Masses of bacteria which are united into mucous clumps by swelling of the capsules (often a sign of death) are called "**zooglea.**"

employed in staining lead to a shedding and degeneration of the flagella, so that their faultless presentation is often a difficult task. (See Technical Appendix.)

The following figures give a schematic view of the three types of equipment of bacteria with flagella. Many pictures of individual varieties are found in the atlas.

In cultures of bacteria with abundant flagella, there at times occurs, as first pointed out by Löffler, a peculiar tuft-like formation of shed or broken-off flagella, intertwined with each other.

The ability to form flagella may be completely lost for generations—whether *permanently* we do not know. Compare Micr. agilis, Sarcina mobilis (Lehmann and Neumann). There have occurred non-motile forms, which were found for months by various authors to be without motion, which later acquired flagella and became actively motile (Lehmann and Zierler). Compare Bac. implexus. Deceptions may thus occur in that many varieties, in spite of being provided with flagella, are only slightly or not at all motile. Also, on the other hand, in many varieties the staining of flagella is so difficult that without anything more certain, a variety cannot be designated as devoid of flagella because it does not move.

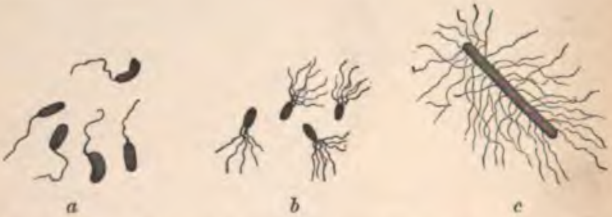

Fig. 7.—Types of flagella: *a*, Vibrio choleræ, one flagellum at the end—**Monotrichia type**; *b*, Bact. syncyaneum, tuft of flagella at the end, rarely at the side—**Lophotrichia type**; *c*, Bact. vulgare, flagella arranged all about—**Peritrichia type.**

The **ordinary vegetative multiplication of bacteria** occurs through transverse fission in the middle of slightly (cocci) or considerably elongated bacterial cells. As a rule, after division, the micro-organisms separate, but the contrary also occurs in the case of bacterial groupings, as where, for example, chains of cocci or bacilli are present. Under definite conditions of nourishment there occurs in bacteria, vibriones, and higher fission-fungi, the formation of longer threads, which further may always again break up into segments. According to all the recent observations, the division of the cells proceeds from the outer protoplasmic layer.

Although longitudinal growth with transverse fission is the rule for the host of bacteria,[1] still in certain families—for example, sarcina—there occurs a regular division in three main planes, and at least occasional division in two planes at right angles to each other is observed in very different bacteria,—for example, in streptococci,—whereby there may be produced cells with four parts and forkings of the chains. (Compare Fig. 2.)

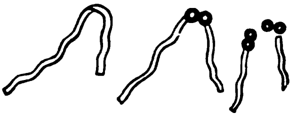

Fig. 8.—Arthrospores of the vibrio choleræ (after Hüppe).

From the ordinary vegetative multiplication, propagation by **spore-formation** is to be distinguished. To-day there are recognized: (1) **Endospores**—strongly refracting oval or round bodies, situated in the interior of cells, which, as a rule, possess considerable resistance to injurious agencies (heat, drying, chemicals); and (2) **arthrospores** (De Bary, Hüppe)—*i. e.*, bud-like constrictions of the ends of the cells. Also these (Fig. 8) should be characterized by increased resistance, yet recent authorities have never acknowledged that the proof of resisting arthrospores has succeeded without objection. (Compare Vib. choleræ and Strept. pyogenes.)

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[1] A longitudinal division in bacilli, although rare, is undoubtedly observed (Babès, Z. H. xx, 412). Stellate division has been observed by Metschnikoff in a sporulating organism named "Pasteuria," but it scarcely belongs among the bacteria in the restricted sense.

In the following, therefore, where the term **spore** is used, only **endogenous resting forms** are to be understood.

The origin of **endospores** proceeds similarly, but not identically, in the individual varieties. For the investigation of a certain variety as to spore-formation, as a rule we employ an agar-streak or potato culture, which has been grown at a temperature near the optimum for the variety. After twelve, eighteen, twenty-four, thirty, thirty-six, etc., hours portions of the *unstained* culture are examined in water with a *narrow diaphragm*. If spherical or oval, strongly refracting spores seem to be present, they must be **stained** according to Neisser or Hauser. (Compare Technical Appendix.) For the *more exact* following of spore-formation it is best to place a few bacilli in a drop of gelatin or agar, and, with the aid of a warming apparatus or in a well-warmed room, to continuously observe and draw definite individuals.

Motile varieties (according to Fischer) always become quiet before sporulation, yet without shedding their flagella. Many varieties grow into longer threads, at first unjointed, before spores form. To these latter belongs the anthrax bacillus, whose sporulation will here serve us as a model. (Compare Plate 36, Figs. III and VI.)

There is first seen a delicate dusty cloudiness in the previously homogeneous bacteria; then, according to Bunge, instead of these finest dust-like particles, a small number of slightly coarser granules appear, which unite among themselves until at regular intervals there lie small round spores (36, VI), which gradually change into the oval, strongly refracting, mature spores (36, III).

When the spore-formation is complete, there is seen in the thread of bacilli a delicate partition-wall between each two adjacent spores (36, IV). Spores are not matured in all the segments, although the globular precursors may have prepared the way for it. Indeed, **some varieties,** *on account of certain gradually introduced cultural conditions,* **lose the property of producing mature spores,** only physiologically worthless antecedents being formed (Roux, K. B. Lehmann).

According to Lud. Klein (C. B. VII, 440), spore-formation is quite different in five varieties of anaerobic bacilli, mostly motile, discovered in swamp-water and studied by him, but unfortunately they were not cultivated in pure culture. (Bacillus De Baryanus, Solmsii, Peroniella, macrosporus, limosus). His observations were as follows: Without loss of motion, the end of the bacillus swells somewhat and becomes faintly greenish. Now the whole content of the swollen spot contracts into a shining spore of a bluish-green color and highly shining.

Mature spores are arranged in the most important varieties in the following manner (Fig. 9): The spore lies inside of an unexpanded short bacterial cell (*a*), or at the extreme end of a bacterial cell (*c*), or inside of a spindle-formed bacterial cell, bulging at its center (*d*), or, finally, the spores occur in a row in a thread, formed from short cells, each cell containing a spore (*b*).

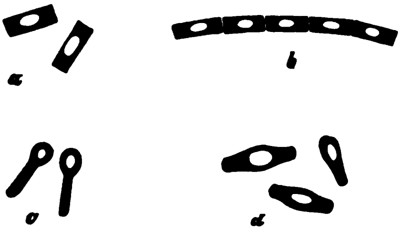

Fig. 9.—Types of spores.

The spores, before germination occurs, are usually free (an exception occurs in spirillum endoparagogicum), often show an indistinct border, always lose their luster, become somewhat thicker, and rarely also longer. Usually after one, two, or three hours the spore membrane bursts and the young rod, sometimes suddenly, sometimes slowly, presses itself through the rent. The germination in **anthrax** is polar—*i. e.*, the young rods leave the spore capsule at or near the pole (Fig. 10). In other varieties (B. subtilis, mycoides, megatherium) the escape of the rod is equatorial (Fig. 10, *a*). Burchard describes also a bipolar and oblique mode of escape. According to the observations of Bunge (Fort. der Med., XIII, 813, 853), in both the polar and equatorially germinating varieties, single or many individuals always present an oblique outgrowth.

This has been completely confirmed by myself and Dr. Hirai in Bac. anthracis, Bac. gangrænosus pulpæ, and Astasia asterospora. From what we have seen, it appears strange to us that Burchard (A. K. II, 1) found twenty-one new species of spore-carrying bacilli, the spores of which all germinated so differently and characteristically that he held the occurrence of the spore germination (appearance of spore, point of germination, thickening of spore capsule, etc.) to be a certain diagnostic aid in differentiating the variety. Until now we unfortunately have not been in a position to confirm the statements upon more extensive material.

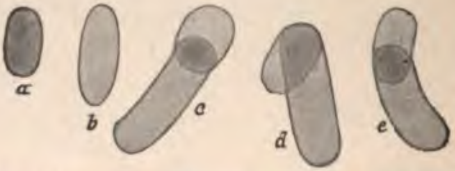

Fig. 10.—Polar germination of spores in anthrax.

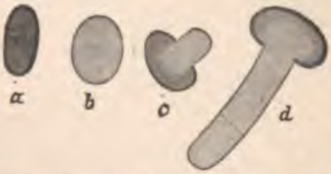

Fig. 10 *a*.—Equatorial germination of spores in Bac. subtilis.

Regarding method, I may remark that spores are allowed to dry in a thin layer on a cover-glass; a drop of agar is placed thereon and the hanging drop examined upon a warm stage.

In old cultures of bacteria there are found almost always dead, often very strangely deformed, bacterial cells, **involution and degeneration forms**, of which Plate 36, Fig. V, and Plate 51, Fig. IV, give an idea. These swollen, bent, often entirely unrecognizable forms stain poorly by ordinary means. The beginner will often mistake involution forms for contaminations. Plate cultures soon determine whether one or more forms of bacteria are present.

B. Chemical Composition of Bacteria.

Qualitatively considered, the **bodies of bacteria**[1] consist in great part of **water, salts,** and **albuminous bodies**;[2] in lesser quantity are present **extractive substances** soluble in alcohol, and others soluble in ether (**triolein, tripalmitin, tristearin, lecithin, cholestearin**). In tubercle bacilli Aronson found in the ethereal extractive (25% of the dry substances), besides free **fatty acids,** large quantities of **wax,** whose alcohol differs from cholesterin. In no variety of bacteria could E. Cramer find **grape-sugar,** although many varieties (Bacillus butyricus, varieties of leptothrix) contain **starch-like masses,** turning blue with iodin. True **cellulose** was found by Dreyfuss in B. subtilis and a bacillus resembling the B. coli; also, the Mycobacterium tuberculosis forms cellulose in the animal body. The vinegar-forming Bact. xylinum produces such a quantity that visiting cards have been made from it as a curiosity. From *cultures* of Myc. tuberculosis and a "capsule bacillus from water" resembling the B. pneumoniæ of Friedländer, on the contrary, no cellulose was obtained, but instead there was found abundant mucoid carbohydrates, $C_6H_{10}O_5$, closely resembling **hemicellulose.** (For literature, see Nishimura, A. H. XVIII, 318, and XXI, 52). Scheibler (Chem. Centralbl. XI, 181) has described the mucus-like material of the *Streptococcus mesenterioides* as a carbohydrate, $C_6H_{10}O_5$, **"dextran"**; Kramer has obtained a similar substance from the capsules of the *Bac. viscosus sacchari.* Up to this time, **nuclein** has not been obtained from bacteria in any quantity; on the contrary, of the nuclein bases, **xanthin, guanin, adenin** have been obtained in considerable quantities. A number of bacteria contain sulphur granules, which have arisen from sulphuretted hydrogen (Beggiatoa thiothrix). Others, still considered as belonging to the bacteria by many authors, deposit **oxid of iron** in their capsules, obtaining it from water containing iron (cladothrix, crenothrix).

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[1] H. Buchner has directed that the cell-contents (bacteria protoplasm) be obtained by trituration and the hydraulic press (3 to 500 atmospheres). Compare Hahn (C. B. XXIII, 86).

[2] Albumin and salts can constitute as much as 98% of the dry bodies of bacteria (Vibrio choleræ); on the contrary, as much as 12% of carbohydrates may be present in the capsules. In bacterial albumin Hellmich recognized a globulin (Arch. f. exp. Pathol. u. Pharmak. XXVI, 345). Most improbable appears the statement of Fermi that he has grown nitrogen-free (!) micro-organisms (C. B. L. II, 505).

Regarding **quantitative composition,** the methodical work of E. Cramer has added considerable light, though up to the present exact statements are submitted regarding only the B. prodigiosum, B. pneumoniæ, and some related organisms, and also a series of cultures of the *Vibrio choleræ.* Compare E. Cramer, A. H. XIII, 71; XVI, 151, and XXII, p. 167.

The **water content** of a culture grown on solid nutrient media, as also the **amount of ash,** depend *in a very large measure* upon the *composition of the medium.*

For example, the B. prodigiosum contained, when grown upon potato, 21.49% dry substance, 2.70% ash in the fresh substance; when grown upon yellow turnip, 12.58% dry substance, 1.31% ash in the fresh substance. Besides the concentration of the nutrient medium, *higher temperature* and *lessened age* of the culture act to increase the dry substance and the ash.

Also the **dry substance** of bacteria varies in its composition under the influence of the nutrient medium.

Thus, for example, the Bact. pneumoniæ Fried. shows upon meat-infusion agar containing

	1% PEPTONE.	1% PEPTONE AND 5% GRAPE-SUGAR.
Albumin	71.7%	63.6%
Ethereal and alcoholic extractives	10.3%	22.71%
Ash	13.94%	7.88%

Evident increase in the proportion of peptone in the nutrient medium leads to an increase in the albumin in the bacteria, while increase of grape-sugar makes the body substance poorer in albumin and increases the alcoholic and ethereal extractives (Lyons; A. H. XXVIII, 30).

Yet there is much greater difference in the dry substance of the cholera vibriones, if they are grown at one time upon soda bouillon rich in albumin, and again upon Uschinsky's medium, free of albumin. Cramer here found as follows (the figures are the average from observations upon five cholera cultures):

	ALBUMIN	ASH
Cholera vibriones on soda-bouillon	65%	31%
Cholera vibriones on Uschinsky's solution . .	45	11

He found also in the latter case a considerable quantity of non-nitrogenous bodies, a part of which may be thought to be hydrocarbons (or fats).

For the analysis of the ash of bacteria, consult Cramer (A. H. XXVIII) and de Schweinitz and Marion Dorset (C. B. XXIII, 993). The latter found almost only phosphate in the ash of tubercle bacilli.

Of importance for the classification, even though more in a critically negative sense, is the fact, discovered by Cramer, *that closely related varieties, which, upon many nutrient media, present analogous slightly varying composition, suddenly upon a new medium conduct themselves differently.* Most interesting in this respect is the behavior of five cultures of cholera, which in soda bouillon furnished vibriones of almost exactly the same composition, but upon Uschinsky's [1] solution presented very variable composition.

	SODA BOUILLON.			USCHINSKY'S SOLUTION.		
	Albumin.	Ash.	Total.	Albumin.	Ash.	Total.
Cholera, old . . .	65.12	31.55	96.67	48.13	7.14	55.27
Cholera, Hamburg I	69.25	25.87	95.12	35.75	13.70	49.45
Cholera, Paris . . .	62.25	32.80	95.05	65.63	9.37	70.00
Cholera, Shanghai .	64.25	33.87	98.12	47.50	11.64	59.14
Cholera, Hamburg II	63.94	29.81	93.75	34.37	14.74	49.11

This result shows again how **dangerous** it is to make a **separation of two varieties** because of **any single** chemical or biological **reaction.** In order to understand the astonishing differences, it is only necessary to recognize the ability of one of these varieties to form thick cell-membranes from Uschinsky's solution. How easy it would be for an author to pronounce the cholera of Paris among this number, as a distinct species, since, upon Uschinsky's medium, *it contains almost double the amount of albumin which the Hamburg cholera does.*

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[1] Compare p. 33.

Bacterial spores have not so far, to my knowledge, been closely studied. One may naturally expect a decreased water-content, from the analogy to the spores of molds.

C. Rapidity of Increase and Duration of the Life of Bacteria.

Under favorable conditions (see below) bacteria multiply very rapidly; according to Buchner, the number of cholera vibriones, under most favorable conditions, is doubled in twenty minutes (C. B. II, 1). Compare also Ficker (C. B. XXIII, 1059).

The duration of the life of bacteria is theoretically unlimited, since from each cell by division two new ones, with unlimited possibilities of division, are produced. Practically, however, in our cultures the case is quite different. As pointed out by Gotschlich and Weigang, in a cholera culture (agar-streak) at 37°, even after twenty-four hours the number of live germs is practically reduced, and after forty-eight hours many bacteria are injured by their own products (Z. H. XX, 376).

D. Conditions of Life of Bacteria.

1. NUTRIENT MEDIA.

While a number of bacteria have hitherto been met with only in the human or animal organism as parasites, and appear to us as **obligate parasites** (example, Spirochæte Obermeieri), yet most of the parasites can be grown upon artificial nutrient media, either readily (example, Bacterium typhi) or with more difficulty (example, Micrococcus gonorrhœæ). Of the inhabitants of the inanimate surroundings of man, *i. e.*, the so-called **saprophytes,** most are easily cultivated on artificial media, similar to those employed for parasites, while others—as, for example, saliva bacteria and certain water bacteria—offer great or in part insurmountable difficulties in their cultivation.

All nutrient media for bacteria must be **rich in water**; the presence of **salts,** and sources for the supply of **carbon** and **nitrogen** are indispensable. Most varieties of practical importance and all pathogenic varieties prefer a medium containing albumin which is faintly alkaline in reaction.

In some cases the **demands of the bacteria** as regards the composition of the nutrient medium are very different. As shown by Mead Bolton, a number of water bacteria (Bacillus aquatilis Flügge and B. erythrosporus Flügge) are contented with water which has been twice sterilized in glass vessels (Z. H. I, 76). Here an increase of the bacteria must occur at the cost of traces of impurities, or of the ammonia and CO_2 of the atmosphere.

Almost simultaneously Heraeus (Z. H. I, 193) observed a variety of bacterium, which thrived in water which contained ammonium carbonate as the only source of carbon and nitrogen, being free from every organic nutrient material. Here, then, there occurred the **elaboration of living substance from simple materials,** just as occurs in the higher plants which work with chlorophyll aided by sunlight. Hüppe and Winogradsky have demonstrated by extensive studies the truth and importance of this observation. It appears that the energy necessary for the synthesis of albumin is obtained by oxidation of ammonia into nitric acid. Among the practically important bacteria, such unparticular ones are very few. Many allow albumin to be absent from the medium and are content with very simply composed nutrient solutions. Cultures upon such fluids were formerly much employed, and more recently Uschinsky has again experimented with simple nutrient solutions. The solution of Uschinsky is as follows:

Water, 1000.
Glycerin, 30 to 40.
Sodium chlorid, 5 to 7.
Calcium chlorid, 0.1.
Magnesium sulphate, 0.2 to 0.4.
Di-potassium phosphate, 3 to 2.5.
Ammonium lactate, 6 to 7.
Sodium asparaginate, 3 to 4.

Instead of this complicated solution, one may employ many simpler ones; for example, such as is recommended by Voges and C. Fränkel (Hyg. Rundschau, 1894, No. 17, 769), which is as follows:

Water	1 liter.
Sodium chlorid	5 gm.
Neutral commercial sodium phosphate	2 gm.
Ammonium lactate	6 gm.
Asparagin	4 gm.

Upon this (although there is no *sulphur* in the nutrient medium) the following grow:

VERY WELL:
Bac. subtilis and mycoides,
Bact. syncyaneum, pyocyaneum, coli, acidi lactici, pneumoniæ, mallei, vulgare,
All vibriones.

FEEBLY:
Mic. pyogenes α aureus,
Streptococcus pyogenes,
Bact. typhi,
Bac. anthracis.

NOT AT ALL:
Bac. tetani,
Bact. murisepticum,
Bact. erysipelatos suum,
Bact. cuniculicida.

Even with the addition of those substances recommended by Uschinsky, other varieties, as diphtheria[1] and tetanus, did not grow luxuriantly, but, by adding 3% to 4% of glycerin, the medium can be used for cultivating many varieties, even the tubercle bacillus.

While cultures upon the simple nutrient media just described possess a great theoretical interest, yet they have been but little employed for diagnostic purposes.

Very much more use is found for *meat-infusion*, *peptone-gelatin*, *agar*, and *bouillon* (*each with or without the addition of grape- or milk-sugar*), *also glycerin-agar*, *milk*, *and slices of potato*. (For preparation see Technical Appendix.)

We must always keep these on hand, since without them no differential diagnosis is possible, and no variety can be considered regularly described which is not tested in its relation to all these nutrient media (with the exception of glycerin-agar).

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[1] Recently Uschinsky has apparently obtained upon his non-albuminous nutrient medium a good growth with the production of toxin in the case of a certain culture of diphtheria.

More rarely the following nutrient media are employed: *Potato water, veal bouillon, fluid and coagulated blood serum, serum-agar, ascites-agar, blood-smeared agar, meat, pieces of bread, potato-pap, rice-pap, cooked or raw eggs.* (See Technical Appendix.)

Uncooked, sterile organs of animals are actually poorer nutrient media for most bacteria than when cooked (Livingood, C. B. XXIII, 980 and 1002). Studies upon nutrient media containing liver, kidney, thymus, adrenal extract, etc., have been carried out, but without giving anything of practical importance. Literature: (Wroblewski, C. B. XX, 528).

2. REACTION OF THE NUTRIENT MEDIA.

As stated above, the great majority of bacteria, especially the pathogenic, prefer a **neutral or faintly alkaline nutrient medium,** and formerly the advice was always given to neutralize the nutrient medium with soda solution, employing sensitive litmus paper as the indicator—*i. e.*, to add alkali until red litmus paper was turned faintly blue.

Every chemist knows that no accurate terminal reaction for the titration of nutrient media containing phosphates is obtained with litmus; that, further, various litmus papers influence the result; and, finally, that the titration is practically impossible with gaslight. As early as 1891, N. K. Schultz had therefore advocated phenolphthalein as an indicator in the titration of agar. He recommended that 8–10 c.c. less of normal sodium hydroxid be added than is required for complete neutralization with the indicator. Such a medium is found to be suited to many bacteria, yet there are others which demand a complete neutralization (C. B. X, 52).

Without having noticed this proposal, I came upon the same idea in 1892, during my investigations upon bread-acids. Often since then, and exclusively since the autumn of 1894, in my institute there has been employed as neutral gelatin (and agar) a medium which contains just so much sodium hydroxid as is required to produce a minimum reddening of phenolphthalein. All the plates in this atlas are prepared with the use of such nutrient media for such cultures. This was done after the investigation of five important bacteria had indicated to us that additions of acids or alkalis did not improve the growth. Since then I have had the great majority of the bacteria described in our atlas systematically studied as to their ability to grow on the following nutrient media, by Dr. Winkler (Dissert. Würzburg, 1896):

1. On "neutral" agar, neutralized with normal soda with the employment of phenolphthalein.

2. On "acid" agar—*i. e.*, on neutral agar to which was added 10 and 20 c.c. of normal sulphuric acid per liter.

3. On a sort of alkaline agar—*i. e.*, on neutral agar to which was added 10 c.c. of normal alkali solution per liter.

The result, as indicated briefly in Table I (at end of book), is that almost all bacteria grow well on these three media.

In every case the nutrient media **neutralized by means of phenolphthalein** as an indicator may be implicitly employed as **universal nutrient media**; moreover, the *virulence* of those varieties tested by us (Bac. anthracis, Bact. coli, Bact. of mouse septicemia and chicken cholera) is well preserved thereon.

This method has the **advantage** over other methods in that it is easily carried out (compare Technical Appendix), and that it represents a very exact point, namely this, where all free acids and acid salts are changed into neutral salts (mono-sodium phosphate into di-sodium phosphate).

Other recommendations—Timpe C. B. XIV, 845; Heim, Lehrbuch, p. 73; Deelemann (A. G. A. XIII, 374)—appear to have no advantage.

If an *acid nutrient medium* is to be employed, we think it best to add 10–20 or 30 c.c. of normal acid to a medium previously neutralized with phenolphthalein. According to Winkler, the first degree of acidity is well borne by almost all bacteria. According to the certainly not superficial reports of Schlüter (C. B. XI, 589), which were substantiated by subsequent publications, many bacteria bear much higher proportions of acid; according to observations in our own institute, as high as 100 c.c. of normal acid per liter.

Nutrient media containing sugar usually favor the production of acid, which, according to Hellström, soon becomes so abundant that the micro-organisms are killed.

Acid nutrient media are to be used for **yeasts** and **molds** and whenever it is wished to isolate a new bacterium from an acid nutrient substance. For **counting** the germs in air, soil, water, milk, etc., a **neutral** medium is always employed.

3. INJURY TO BACTERIA BY CHEMICAL SUBSTANCES.

We have already learned that too large a proportion of either acid or alkali[1] interferes with growth, or, if still stronger, produces death. Most varied chemicals, in certain concentrations, operate similarly. Those which are strongly active are called **antiseptics** or **disinfectants.**

Usually, with Hüppe, the following grades of influence are distinguished:

1. Growth is not interfered with,[2] but the pathogenic or zymogenic functions are weakened: **Weakening, attenuation.**
2. The organisms are unable to increase, but are not killed: **Asepsis.**
3. The vegetative forms of the micro-organisms are destroyed, but not the resting forms: **Antisepsis.**
4. Vegetative forms and spores are both killed: **Sterilization or Disinfection.**

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[1] Fermi (C. B. XXIII, 208) has published an extensive table regarding the sensitiveness of various micro-organisms to acids, alkalis, and various poisons. Unfortunately, he gives the number of drops of solutions of various percentages which inhibit the growth of bacteria in 5 c.c. of agar.

[2] At times there occurs a transitory or permanent interference with growth; in other cases briefly acting antiseptics, also heat, cold, etc., cause a retardation of subsequent growth without producing a weakening.

Since the diagnostic value of the test of the resistance to chemicals plays only a modest rôle—various hopes in this direction remaining unfulfilled—this section must be very brief.

To determine the *minimal concentration* of a chemical poison which produces **asepsis**—*i. e., prevents growth—the following procedure is adopted:*

A solution of the disinfectant—for example, 1%—is employed; and of this, 1, 0.5, 0.3, 0.1 c.c. is added to 10 c.c. of liquefied gelatin. This nutrient medium, containing now 0.1, 0.05, 0.03, 0.01% of the disinfectant, is used for stab, streak, and plate cultures. Inoculations may also be made with material containing only spores (material freed from all bacilli by heating half an hour at 70°), and thus it may be determined whether the spores grow out in cultures.

Behring has devised the following practical method of making these tests: A drop of fluid nutrient medium,—for example, serum,—infected with the organism to be tested, is removed before the addition of the antiseptic and suspended from the under side of a cover-glass on a hollow-ground slide, sealing it with a little vaselin (Technical Appendix). Then by degrees there is added to the tube of serum, increasing known quantities of the disinfectant. After each addition and thorough shaking a drop culture is prepared. The growth in each drop can be examined after being kept twenty-four or forty-eight hours in the incubator.

If the concentration necessary for **antisepsis** is to be determined, the organisms to be examined are grown in bouillon, and to 10 c.c. of the bouillon, free from spores, and filtered through asbestos to remove any clumps of bacteria, various quantities of a solution of the disinfectant of known strength are added. From each of the tubes after one, five, ten, fifteen, thirty minutes, one hour, etc., a platinum loopful of the material is removed, and placed in 10 c.c. of lukewarm liquefied gelatin, from which a plate culture is prepared. If it is suspected that a trace of the disinfectant carried in the drop renders the gelatin aseptic and so leads to an apparent death of the microorganisms, the result may be controlled by inoculating gelatin, to which a similar trace of the disinfectant has been added, with fresh material.

The disinfectant to be tested should always be dissolved in water. (Compare Water, p. 40.) If, because of slight solubility in water, the employment of *alcohol* in the preparation of the stock solution is unavoidable, then special control investigations are required to determine whether the alcohol is injurious in its effects.

It is found, as well for asepsis as for antisepsis, that the value of the disinfectant is usually *much* **lower** if one is working with nutrient media **rich in albumin** than when working with those poor in albumin.[1] Creolin (Pearson) produces asepsis in bouillon in the proportion of 1:15,000 to 1:5000, but in beef serum in 1:150 (Behring). Cholera vibriones, in bouillon free from or containing 1% of peptone, were killed by 0.01% HCl in half an hour, with the addition of 2% peptone by not less than 0.04% HCl in the same time. For descriptive purposes, the test will usually be made in 1% peptone solution, if one does not wish to employ one of the nutrient media free from albumin, described on page 34. In any case one will treat the bacteria used for comparison exactly the same, and must give in a paper the finer details employed in the investigation. Little is known of varying resistance in bacteria which are free of spores, because of race or nutrient media (compare spores), but there are isolated statements in this direction regarding staphylococci which, perhaps, may point to as yet imperfectly understood resting forms (Esmarch, Z. H. v, 67).

A combination of disinfectants may enhance the action; the addition of acid (hydrochloric or tartaric acid) especially intensifies the action of sublimate, also of phenol and cresol solutions. Moreover, the effect is more certain if the germs are few than if they are abundant, and greater at higher than at lower temperatures.

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[1] Phenol is an exception.

4. DEFICIENCY OF NOURISHMENT AND WATER.

If bacteria which require substrata rich in nourishment in order to thrive (including most pathogenic) are placed in pure **distilled water,** they usually die rapidly—*i. e.*, in an hour. As Ficker (Z. H. XXIX, 1) pointed out, numerous minute influences here become accentuated. Thus, traces of nutrient material, even long standing in glass vessels, especially boiling in glass vessels, suffice to lessen the bactericidal action of distilled water. Jena glass is especially recommended for control investigations, since it gives up almost nothing to distilled water. Dense suspensions and virulent cultures are more slowly destroyed; the age of the individuals is indifferent. In **well-water** (even if sterilized) the duration of life is usually not more than eight to fourteen days, and an increase is rare. Certainly, in a series of cases, much longer duration of life is observed, but here the conditions mentioned above, and until now usually neglected, come into question. Leipzig tap-water, which remains long in pipes, is strongly germicidal, but after being boiled it loses a part of this action, etc. (Compare Ficker, *l. c.*, and Löffler: Das Wasser und die Mikroorganismen, 1896.) **Deficiency of water** exerts a disturbing influence upon bacterial growth. My pupil, Leo Wolf (A. H. XXXIV, 200), found that upon various nutrient media (agar, gelatin, pulverized meat, cake), with 70% and more of water, growth was most vigorous, with 60% it was often interfered with, with 50% it was usually slight, and when only 40% of water was present there was often no growth to be detected, macroscopically at least. On the contrary, upon nutrient media (agar, gelatin, potato) **dried** gradually at room temperature, the **duration of life** is often astonishingly long, even when there are no endospores present to be responsible for it. At times one may observe that even after a year a horny contracted remnant of a culture upon being placed in bouillon (and with proper varieties kept in an incubator) yields a most beautiful growth.

Regarding the **duration of life of bacteria,** when they are spread upon glass and **dried,** the literature contains many contradictory statements, from which it may be concluded that much depends upon the special conditions under which drying occurs. Ficker (Z. H. XXIX, 1) has discovered some laws especially for the cholera vibrio.

According to him, the following are of special significance:

1. Very thin smears were more quickly injured than thick clumps; slight variations in the thickness of the smear are without significance.
2. Thin smears die more quickly in the exsiccator, and thick ones in room atmosphere.
3. The lower the temperature, the better the drying is borne.
4. Virulent cultures are more resistant than non-virulent ones.
5. Variations of moisture (exsiccator and moist chamber) kill especially rapidly (shown in typhoid and pest).
6. Older cultures were somewhat more resistant in an exsiccator.

Also regarding the variability in a moist chamber, Ficker has obtained interesting results. Here old cholera cultures present enormously greater resisting power than young ones. For example, in the moist chamber thin smears of cultures seven to twenty-one days old live about thirty to fifty days; those three days old, fourteen days; those two days old, as long as seven days; and those one day old, only one to two days. Yet the old cultures contained no spores, and were equally or more susceptible than the young ones to heat and chemicals. (Compare Vib. chol. in special part.) A complete summary of previous results upon dried cholera, typhoid, diphtheria, and pest organisms is given by Ficker, *l. c.*

5. RELATION TO OXYGEN AND OTHER GASES.

Because of their relation to oxygen, bacteria are usually placed in three classes (Flügge and Liborius):

I. Obligate aerobes. Growth occurs only when *air is admitted*, and every limitation to the entrance of air injures the growth. Free oxygen is especially required for spore-formation. With a pressure of three to four atmospheres of pure oxygen, according to Chudiakow, growth of aerobic bacteria ceases; the lowest limit for aerobes was found to be 5 to 10 mm. of air. According to Roger (C. B. XX, 626), a pressure of 500 to 600 atmospheres of air does not kill bacteria.

II. Obligate anaerobes. Growth and spore-formation occur only when *oxygen is completely excluded.*[1] In this class belong Bacillus œdematis maligni, Bac. tetani, Bac. chauvœi (symptomatic anthrax), and numerous inhabitants of mud and soil. Upon exposure to free atmospheric oxygen, the vegetative forms of these bacteria die in one or more hours; the spores, on the contrary, are more resistant to oxygen. (Compare Chudiakow, C. B. L. IV, 389.) Since the principal source of energy is excluded from the anaerobes which is at the command of aerobic bacteria (oxidation of the absorbed nutrient material by means of free oxygen), they are assigned to nutrient materials which are readily utilized,—as, for example, grape-sugar,—which liberate energy (heat) by division into two smaller molecules (for example, alcohol and CO_2, or acetic acid, or lactic acid). Therefore anaerobes are often grown upon gelatin or agar containing 1% to 2% of glucose. Upon such media the virulence and also the ability to produce spores suffer, so that recently sodium sulphid (compare p. 43) and formate of sodium have been employed as additions to the culture media.

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[1] Regarding the means of preparing anaerobic cultures consult the Technical Appendix. Chudiakow found that the strictest anaerobes

III. Facultative aerobes and facultative anaerobes.[1] The great majority of the bacteria usually grown by us aerobically—including almost all pathogenic forms—will tolerate a limitation in the amount of oxygen admitted without being injured, moreover without interference with their growth. This is favorable to a life in many parts of the animal body; for example, in the intestinal canal, where oxygen is limited or absent. Chromogenesis is almost always suspended by the exclusion of oxygen; on the contrary, toxic metabolic products are sometimes abundantly elaborated in its absence (Hüppe).

It is very important, as recent inquiry has shown, that *aerobic varieties of the anaerobic bacteria also exist.* Their

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still grew with an air pressure of 5 mm.; three of the best-known anaerobes he found still able to grow with the barometer at 20 to 40 mm. (C. B. L. IV, 389).

[1] As emphasized by Beijerinck, it would be better to speak of "temporary" anaerobic varieties, if it has not been demonstrated that certain varieties can grow *permanently* as well aerobically as anaerobically (C. B. L. III, 40). The demonstration produced by Pfeffer is also interesting, that many aerobic bacteria can bind loosely considerable amounts of oxygen, which they gradually release in spaces free from oxygen (C. B. L. II, 763).

origin is usually not known. (Compare special part, Bac. tetani, Bac. chauvœi.)

Not infrequently varieties are observed which show more or less anaerobic growth when first isolated (growing especially in the deep part of the agar stab), and which later present a pure aerobic behavior—*i. e.*, distinct surface growth and poor growth along the stab. Thus, one may properly speak of an **adaptation** to a gas-mixture, rich in, or free from, oxygen, and in this way may explain very many things.

These observations prove to the classifier that bacteria can not be thus simply separated into two classes, the one aerobic and the other anaerobic.

Recently the fact has been repeatedly demonstrated that true anaerobic varieties also grow well without the exclusion of oxygen, if they are associated with many aerobic varieties; also that for the aerobic growth of anaerobic varieties, often nutrient media suffice if aerobic bacteria have previously grown in them and have been killed with chloroform before the anaerobe is inoculated (Kedrowski, Scholz). The synergetic aerobic variety evidently operates in part by consuming the oxygen, in part by producing materials required by the anaerobic varieties. Trenkmann recognized such a substance in sodium sulphid. Two drops of a 10% solution of sodium sulphid render bouillon suitable for the growth of anaerobic varieties without the exclusion of oxygen (C. B. XXIII, 1038).

While, besides the obligate anaerobes, all *facultative anaerobes* thrive well in nitrogen and hydrogen, they vary in their toleration of CO_2. (Compare Table I, at end of book.)

A large number do not thrive at all, and remain completely inhibited in their growth until oxygen is again admitted; for example, B. anthracis, subtilis, and related varieties. It is established regarding some varieties (anthrax, cholera) that most of the individuals are rapidly killed by CO_2, while some germs exhibit energetic resistance, and render a complete sterilization by CO_2 impossible. A second group presents a restricted growth, especially if the test is carried on at incubator temperature (staphylococci, streptococci), while a third group is not injured at all: B. prodigiosum, B. acidi lactici, B. typhi. They grow as well as in air; the liquefaction of gelatin is not interfered with, but naturally, from the exclusion of oxygen, chromogenesis is checked. Moreover, a *mixture* of 25% air and 75% CO_2 has no apparent injurious influence upon fungi, which remain absolutely undeveloped in an atmosphere of pure CO_2 (C. Fränkel, Z. H. v, 332).

Sulphuretted hydrogen appears to be well borne by anaerobes (see above); other varieties are very susceptible to large quantities, as, for example, Bact. Pflügeri (photogenic bacillus) (Lehmann and Tollhausen, C. B. v, 785).

6. INFLUENCE OF TEMPERATURE ON THE LIFE OF BACTERIA.

Every variety of bacterium demands a certain **temperature** of the nutrient substratum. Vegetative bacterial life is possible from 0° to 70°, some varieties thriving at the upper and some at the lower extreme. For each variety the minimum and maximum temperatures lie about 30° apart,[1] and we may form a comprehensive classification dependent upon the temperature required somewhat as follows:

Psychrophilic bacteria: minimum at 0°, optimum at 15° to 20°, maximum at about 30°. Most water bacteria belong here; for example, many phosphorescent bacteria of the sea. (Compare Forster, C. B. XII, 431.)

Mesophilic bacteria: minimum at 10° to 15°, optimum at 37°, maximum at about 45°. Here belong all varieties pathogenic for man, since one condition for a pathogenic action is an acclimatization to the body temperature.

The B. vulgatus connects this and the following group, as it still grows well at 50°.

Thermophilic bacteria: minimum, 40° to 49°; optimum, 50° to 55°; maximum, 60° to 70°. Here belong many bacteria of the soil, and almost all spore-producing bacilli of the family of B. mesentericus (Globig, Z. H. III, 294).

More lately Lydia Rabinowitsch has somewhat more closely described eight thermophilic facultative anaerobes, all of which are spore-producing, non-motile bacilli, whose optimum temperature lies at 60° to 70°, but they still thrive at 34° to 44°, although slowly, and best in anaerobic agar cultures (Z. H. XX, 154). The varieties are widely distributed, especially in feces. She has not undertaken a comparison with the varieties described by previous authors. Other varieties were isolated by Oprescu (A. H. XXXIII, 164). Some of the varieties isolated by Schillinger appear more as abnormally thermotolerant than thermophilic; they grow well at 66°, but better and with fermentation at 37° (H. R., 1898, 568).

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[1] Bac. vulgatus thrives, to be sure, from 15° to 50°, a variety of Globig also from 15° to 68°, but such wide intervals of favorable temperature are very rare. Globig found the range of temperature at which thermophilic varieties will develop to be very narrow; for example, he could grow one variety only from 54° to 65°.

By carefully raising and lowering the temperature, Dieudonné (C. B. XVI, 965) was able to increase the **range of temperature** within which the *anthrax bacillus* could thrive, at both its upper and lower limits. The anthrax bacillus can gradually become adapted to a temperature of 42°. Pigeons, which, according to the hypothesis of many authors, are fairly immune to ordinary anthrax because of their higher body temperature (42°), die somewhat oftener after inoculation with cultures adapted to higher temperatures. Still more striking were the results when Dieudonné gradually acclimated anthrax bacilli to a temperature of 12°, and found they could still kill frogs kept at 12°.

Temperatures *somewhat* **below the minimum** limit the growth, but do not injure the variety concerned. Petruschky has even kept bacteria in an ice-box (about 4° to 6°) as a means of preserving not only the life but also the virulence of certain varieties which rapidly die at higher temperatures. They are first allowed to grow for two days at 20° (streptococci, etc.).

Also **temperatures below zero** injure bacteria, but do so slowly and with a rapidity varying with different varieties. Individual statements are given in the special part regarding the most important pathogenic varieties.

If **temperatures 5° to 10° above the optimum** are allowed to operate upon cultures, they are injured in various ways; some show lessened intensity of growth, the virulence and the power of fermentation are reduced, also the ability to form spores is gradually lost. Sometimes the injury is more marked in one direction, sometimes in another.

If the **maximum temperature is exceeded,** the culture dies, and for the psychrophilic varieties about 37° is quite rapidly fatal; for the mesophilic,[1] about 60° (Forster); for the thermophilic, 75°. A temperature of 100° is not withstood by any bacterium free of spores for more than a few minutes.

7. MECHANICAL AND ELECTRICAL INFLUENCES.

In the first edition, at this point I reported the astonishing statements of Meltzer, according to whom short, feeble shaking would operate favorably upon the growth of fluid cultures of bacteria, while more prolonged and more vigorous shaking or long-continued very feeble shaking would operate very unfavorably (Meltzer, Zeit. f. Biol. XXX, p. 464).

Otto Appel, who, at my request, restudied the whole question, arrived at entirely different results. No shaking of longer or shorter duration injured the bacterial growth, except where very severe agitation and the addition of glass pearls caused direct mechanical lesions of the bacteria. Slight shaking, such as cultures experience when placed upon the foundations of strongly working steam-engines, was without effect. (Further communications thereon are found in the Archiv für Hygiene.)

Most of the previously observed effects of the electric current are easily explained as due to the action of heat and electrolysis. Thiele and Wolf demonstrated by means of new investigations, which are not open to objection, that neither the passage of a constant or interrupted current through a culture of bacteria, if electrolysis be avoided, nor the placing of a culture in an interrupted current induction coil, injures the bacteria (C. B. XXV, 650); *l. c.*, also the previous literature, which in part contains remarkable assertions.

8. INFLUENCE OF LIGHT AND RÖNTGEN RAYS.

The cultures of all bacteria are restricted in their growth by direct **sunlight.** If the action is more prolonged, they are subsequently less able to grow luxuriantly in the dark, and there results a generation of weakened organisms, shown, for example, by incomplete liquefaction, slight production of pigment, lessened virulence, etc., which only regain their original properties after repeated transplantation on fresh medium in the dark. With still longer action of the direct sunlight the micro-organisms die.

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[1] According to Sternberg the following die at 56°: Streptococcus pyogenes, Bac. anthracis, Bact. mallei, and Vibrio choleræ (Amer. Jour. Med. Sciences, July, 1887, 146).

Bact. putidum and Bact. prodigiosum were materially disturbed in their ability to produce pigment and trimethylamin by direct sunlight, in July and August in one-half hour and in November in one and a half hours. They grew slowly and prodigiosum liquefied slowly (Dieudonné). Death was produced in these organisms in one and a half and two and a half hours.

Dieudonné (A. G. A. IX, 405 and 537, also an extensive review of literature) has found ultra-violet, violet, and blue light to be very injurious, green but slightly, and red and yellow not at all. On the contrary, Beck and Schultz (Z. H. XXIII, 490), who employed better light-filters, generally observed no injury from the colored light obtained from sunlight. This is to be explained by the slight intensity of the light employed. Also, Beck and Schultz deny that diffuse daylight works injury to bacteria (the chromogenic function is lost in many varieties even when grown in the dark), while Dieudonné asserts :

In diffuse daylight, in spring and summer in three and a half hours and in winter in four and a half hours, there occurs interference with growth, while in from five to six hours death is produced. Electric arc light of 900 candle-power checks growth in five hours and kills in eight hours. Incandescent light injures growth in from seven to eight hours, and kills in eleven hours. Similar results occur with Bact. coli, typhi, and B. anthracis. Naturally, Dieudonné's positive results are not disproved by Beck and Schultz's negative ones.

For **testing the sensitiveness to light,** thickly sown gelatin or agar plates are exposed to diffuse or direct sunlight, after the method of H. Buchner, a dark paper cross being placed upon the illuminated side. To exclude the action of heat,[1] one may first carry the light through a layer of water or alum a few centimeters in thickness. After the light has acted for one-half, one, one and a half, two, etc., hours, the plates are placed in a dark room, and it is observed whether the bacteria develop only on the part covered by the cross ; in complete death of the exposed bacteria there occurs a sharply outlined cross consisting of the colonies in a clear field.

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[1] The action of the heat is entirely without interest.

The action of the light seems to occur under the cooperation of the **oxygen of the air;** obligate anaerobes (tetanus) and facultative aerobes (B. coli), when oxygen is completely excluded, withstand the sunlight very well; for example, B. coli withstood direct intense sunlight for four hours. (Compare also Wesbrook, Journal of Pathology and Bacteriology, IV, 352.)

Regarding the **mechanism of the action of light,** the observations of Richardson and later of Dieudonné appear important, if not furnishing a complete explanation. They assert that in illuminated agar plates, and indeed only in blue to ultra-violet light, in a short time (even after ten minutes in direct sunlight) **peroxid of hydrogen**[1] appears. For its demonstration one exposes to the light an agar plate, half covered with dark paper, then pours over the same a weak iodid of potassium paste, and upon this a weak solution of ferrous sulphate; the illuminated side becomes dark blue. (With gases that do not contain oxygen there is no formation of H_2O_2, nor injurious action from light.) This also explains what has been often observed, that one may obtain a slight attenuation of bacilli if they are inoculated upon agar plates that have previously stood in the sunlight.[2] Bacteria previously exposed to light develop especially badly upon media that have been illuminated, much more so than upon good media.

According to Rieder, strong Röntgen rays injure bacterial growth in a way similar to light (Münch. med. Woch., 1898, No. 4, 101).

9. THE EFFECTS ON BACTERIAL GROWTH OF OTHER BACTERIA.

Although it is the endeavor of every bacteriologist to always obtain bacteria in pure culture, we must not forget that in nature **bacteria** often occur in **combination.** If we examine water, milk, or the intestinal contents in health or disease, we always find many varieties simultaneously present. This mixture certainly appears to us as a pure accident, but upon closer study it is found that also in the domain of bacteriology there are **synergists** (mutual or one-sided aid) and **antagonists** (mutually injurious, or one to the other). Nencki speaks of **symbiosis** and **enantobiosis.**

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[1] With gelatin it is hours before H_2O_2 can be recognized.

[2] Also other decompositions of the nutrient medium by sunlight may occasionally render difficult a subsequent growth of fungi; for example, the origin of formic acid from tartaric acid (Duclaux).

Experimentally, Garrè has demonstrated the antagonism by inoculating various bacteria simultaneously in streaks upon gelatin plates as parallel or intersecting lines. It then appears that many varieties thrive but slightly or not at all if another variety grows in the immediate neighborhood. The antagonism is very often only on one side; for example, the Bact. putidum grows very well if inoculated between closely placed, well-developed streaks of staphylococci; on the contrary, the Micr. pyogenes does not grow if inoculated between luxuriantly developed cultures of Bact. putidum, and if the two are simultaneously inoculated in alternating streaks, the former grows very slightly (Garrè, "Correspondenzbl. f. Schweizer Aerzte," 1887, 387).

Another way of showing antagonism is by preparing plates of gelatin or agar (for liquefying varieties), which, while liquid, are inoculated with equal quantities of two different varieties of bacteria; often only one variety will develop (Lewek, C. B. VII, 107).

A third way of carrying out the investigation is to inoculate simultaneously the same fluid nutrient medium with two varieties, and, later, determine microscopically or by plates which is triumphant in the battle. This is what is commonly observed when a cause of fermentation is abundantly introduced into a suitable nutrient medium; the contaminating bacteria are overgrown and sometimes perish.

From these observations the practical conclusion is reached that *for determining the number of bacteria in a material the colonies in the plates must not be very thick, and also that for the isolation of definite varieties, thin plates are necessary;* for example, if one wishes to isolate the Bact. Pflügeri from abundant Bact. putidum. In an area of several millimeters about each putidum colony no Bact. Pflügeri will grow (K. B. Lehmann).

Finally, in the **animal body** bacteria may counteract each other as **antagonists**; as Emmerich has pointed out, animals infected with anthrax may be saved by subsequent infection with Streptococcus pyogenes. Literature by Mühlmann (C. B. xv, 895).

Symbiosis of bacteria appears to be of more practical importance, and of this the following examples may be cited :

1. Some bacteria thrive much better together with others than when alone. Some anaerobes thrive even with the admission of oxygen, if only certain aerobic varieties are present. (Compare B. tetani.)

2. Certain chemical transformations—for example, the breaking up of nitrite with liberation of free nitrogen—are not accomplished by many bacteria alone, while two varieties jointly may do so. This fact is well worth considering when searching for the cause of certain decompositions. *Always when the isolated varieties, acting singly, operate partially or not at all, the effects of combinations must be investigated.*

3. In a similar manner it is observed that, for example, the single individuals of a series of soil bacteria are not pathogenic, while certain combinations, inoculated into animals, make them sick.[1] (Liermann, C. B. VIII, 364.) This experience also demands that especial care be taken in searching for the cause of a new or puzzling disease-picture. Many authors believe that cholera has its origin in two germs, "*diblastic theory*" (Nägeli, Buchner).

4. Attenuated pathogenic varieties—for example, attenuated tetanus bacilli—may increase in virulence when cultivated together with other bacteria; for example, Bact. vulgare.

E. The Conditions of Spore-formation and Spore-germination.

The extent of the formation of endogenous spores appears hitherto to have been very insufficiently understood. Except in a large group of important varieties of bacilli, related to the B. anthracis and the B. tetani, undoubted endogenous spores are known only in Sarcina pulmonum, and the strange Spirillum endoparagogicum.[1]

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[1] Not quite appropriate here is the experience that the metabolic products of one variety of bacterium, under some circumstances, may enhance the action of another variety; for example, the metabolic products of the Bact. vulgare the action of the tetanus bacillus.

As H. Buchner (C. B. VIII, 1) pointed out, sporulation occurs in suitable varieties when the nutrient medium begins to be exhausted, therefore **most rapidly** on **nutrient media very poor in nutrient materials.**

On the contrary, a **good nutrient medium** not only favors the growth of bacilli but also the formation of spores, in so far as the vigorously growing bacilli also **luxuriantly** and regularly sporulate (K. B. Lehmann and Osborne, A. H. XI, 51); see especially also Stephanidis (A. H. XXXV, 1). The crop of spores is exceedingly large. The *quality* (resistance) of spores which are grown upon various nutrient media was not found by Stephanidis to vary. For many details consult Schreiber (C. B. XX, 353).

For sporulation a **higher temperature** is sometimes (always?) required than for the vegetative growth. The anthrax bacillus, for example, thrives at 13° to 14°, but does not form spores below 18°.

All *aerobic* bacteria require, especially for spore-formation, the **presence of oxygen**; how the **facultative** anaerobes conduct themselves in this respect is still to be learned.

Obligate **anaerobes** only produce spores if **oxygen is excluded** or, with the admission of oxygen, in mixed cultures or in association with dead synergetic bacteria.

Spores never **germinate** in media in which they have developed when they have been exhausted or rendered detrimental by metabolic products. Only after transferring to fresh nutrient media does germination occur, appearing in one or more hours, and having the morphologic peculiarities described on page 26.

Against all injuries spores are substantially more **re-**

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[1] As it is important for our classification, we have carefully sought, in a number of varieties generally considered as being free from spores, to obtain spores as had been done by Migula (Sys. I, 207) by means of quince and marshmallow decoction. We never obtained a perfectly undoubted result. With Bacterium janthinum alone we saw detached pictures, which could be interpreted as spores, but we have not studied their germination. Upon the common nutrient media we have not once seen sporulation in a variety commonly known as not possessing spores.

sistant than the vegetative forms. They require no nourishment and no water in order to retain their ability to germinate after years and often decades.[1] They are more indifferent to gases than the bacilli, the spores of anaerobic varieties usually bearing free oxygen well.[2] Spores are obtained by carefully removing sporulating agar streak cultures, and warming the emulsion, prepared with a little water, to 70° for five minutes.

Very important is the resistance of spores to dry and moist heat. Dry heat is especially well borne, a temperature of 100° being withstood by many spores for a long time. In a moist condition, a temperature of 70° kills the anthrax bacillus in one minute; on the contrary, anthrax spores withstand this temperature for hours; even in boiling water or live steam at 100° they die only after two to five, or at times after seven to twelve, minutes. The varying resistance of different anthrax spores (v. Esmarch, Z. H. v, p. 67; Stephanidis, A. H. xxxv, 1) appears to be partly a race peculiarity, but very probably also the nutrient medium, the temperature at which they were produced, the degree of maturity, etc., exert an influence upon the resistance. Very accurate investigations upon these points are almost entirely lacking. We only know from Percy Frankland that spores formed at 20° are more resistant to light than those originating at incubator temperature (C. B. xv, p. 101).

The **resistance of spores is tested** by hanging in the boiling steam-chamber little sacks of tulle containing fragments or little plates of *glass* upon which anthrax spores have been dried, and from minute to minute a sack is removed and the pieces of glass laid upon an agar plate, which is then kept at incubator temperature. A better way it seems to me is as follows: 1c.c. of an emulsion of spores is placed in 20 c.c. of water, and after shaking well five samples of 2 c.c. each are removed and placed in reagent-glasses of equal thinness, while in a sixth one are placed 2 c.c. of water and a thermometer. All six glasses are now plunged in a large water-bath containing boiling water, and after two minutes the thermometer in the control tube reaches a maximum temperature (99° to 100°). Two minutes later one removes the first sample, four minutes later the second, etc., cools them rapidly in cold water, and utilizes 1 c.c. and ½ c.c. of each sample in the preparation of plates. For further details, see Stephanidis, A. H. xxxv, 1.

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[1] According to an observation of v. Esmarch, if anthrax spores are kept a long time the virulence appears to be reduced before the power to vegetate is affected.

[2] Spores of malignant edema in garden earth were well preserved in my institute for four years. On the contrary, very astonishingly, tetanus spores dried upon threads and kept in the room were still alive after two days, but dead after three days.

The varying resistance of apparently identical anthrax spores is of *great practical importance:* (1) in disinfection experiments, which should be carried out with spores of known resistance; (2) in differential diagnosis, as it indicates how very careful one must be in placing dependence upon a slight difference in the resistance of spores in determining two species.

Very extraordinary is the resistance of many varieties occurring in hay and soil. Christen found, for example (C. B. XVII, p. 498), that with compressed steam the resisting spores in soil were killed—

At 100° in more than 16 hours.
" 105°-110° in 2 to 4 hours.
" 115° " 30 to 60 minutes.
" 125°-130° " 5 minutes and longer.
" 135° " 1 to 5 minutes.
" 140° " 1 minute.

The apparatus employed brought the objects to the desired elevation of temperature very quickly.

Also against *chemical agents* spores are very resistant; thus, anthrax spores (v. Esmarch, *l. c.*) resist 5% carbolic acid for at least two days, and in many cases as long as forty days. Very resistant anthrax spores withstood a 1% aqueous sublimate solution for three days, but the virulence is lost in twenty hours. Such experiments are best made with thin suspensions of spores in water, to which the disinfectant is added, just as was pointed out above for testing the action of antiseptics upon bacteria (p. 38).

In testing the resistance of spores to gases, they are best dried upon pieces of glass, and the gas allowed to operate in a dry and also in a moist chamber (compare p. 41).

Spores are also less injured by light than bacilli are. As with bacilli, an atmosphere containing oxygen is necessary in order for injury to occur. According to Dieudonné, anthrax spores upon agar plates were dead after an exposure to direct sunlight for three and a half hours (bacilli in one and a half hours); if oxygen was excluded, an exposure of nine hours produced no injury.

F. The Activities of Bacteria,

Especially in Regard to the Application of the Same to Diagnostic Purposes.

The activities of bacteria in the test-tube may be designated[1] as: (1) **mechanical,** (2) **optical,** (3) **thermal,** (4) **chemical.** They will here be discussed in this order; a fifth section will show how the activities of the bacteria enable them to become the **causes of disease** (pathogenic action).

All the **activities** of a given variety of bacterium are especially **dependent** upon, (1) *the momentary condition of the bacterium;* (2) *the nutrient substratum;* (3) *the entrance of air;* (4) *the temperature;* (5) *the illumination.*

Since we have already stated what is most important regarding the influence of temperature and light, in the following I must especially discuss *the influence of the nutrient medium* and *the admission of air* on one side, and the *composition of the final culture* on the other. The latter point must always be made especially prominent, in order to show, in the largest possible range, *how very much the activity of bacteria changes, according as they are examined when in a full zymogenic, chromogenic, or pathogenic condition, or in an attenuated state.*

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[1] It is self-evident that to-day a division of bacteria into zymogenic, saprogenic, chromogenic, and pathogenic is not acceptable. Bact. coli causes, for example, in sugar-solutions powerful fermentation; on nutrient media rich in albumin it produces abundant indol and sulphuretted hydrogen; it forms upon potato very often a rather bright brownish-yellow colored layer, and is besides pathogenic for animals and man; it therefore combines the properties of all four groups.

1. MECHANICAL ACTIVITY.

Under the microscope we easily observe that many bacteria present pronounced **inherent motion,** and by study it is found that almost all the motile varieties[1] possess **flagella** by means of which they propel themselves. The character of the motion is exceedingly variable; for example, creeping (B. megatherium), wabbling (B. subtilis), rolling, snake-like (vibriones) ; sometimes it is very slow, and sometimes so rapid that any detailed observation can scarcely be made (B. typhi).

In many cases it is difficult to decide whether true **active motion** is present, or whether the micro-organisms present especially well-marked **Brownian** or **molecular motion**—*i. e.*, the dancing and tremor exhibited also by finely divided inorganic particles. In such a case it is recommended to try to render the flagella visible by staining (Technical Appendix), and also to examine the organism in a drop of 5% carbolic acid or 1 : 1000 sublimate solution, when, if the motion still persists, we have only to do with molecular motion. Many varieties appear on brief observation to be quiet, but on longer examination single individuals are observed to exhibit positive motion. It seems that the endowments with flagella and motility, when once present, are for the most part reasonably constant peculiarities. Many varieties do not always present motility, it being absent, especially, on many media. According to A. Fischer, with faultlessly developed flagella motion may be absent; for example, in Bac. subtilis on a nutrient medium containing 2% to 4% of ammonium chlorid. We have never observed spontaneous motion or flagella in two different cultures of the Micrococcus agilis Ali-Cohen, obtained from reliable sources and grown upon all ordinary media. We have arrived at the conviction that the same variety may occur either with or without flagella. (Compare special part.)

Th. Smith has described a non-motile form of the motile hog-cholera bacterium ; and motile pest cultures and motile bacteria of septicæmia hæmorrhagica have been described in isolated instances. Compare also what is said in the special part regarding the Bac. implexus.

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[1] Upon the actively motile *Spirochæte Obermeieri* and the slowly creeping *Beggiatoa* no flagella have thus far been demonstrated; therefore the motion is supposed to depend upon a narrow **undulating membrane** attached to the organism.

As first shown by Pfeffer, many chemical substances actively attract (**positive chemotaxis**) and others repel bacteria (**negative chemotaxis**). Oxygen is particularly attractive for aerobic and repellent for anaerobic bacteria. Like Beijerinck, one may obtain very beautiful **chemotaxic** or **aerotaxic figures** in the following manner : An unsterilized pea or bean is placed in a test-tube which is three-quarters full of sterile water. The bean gives off nutrient materials by diffusion, which slowly extend upward. In this weak nutrient material certain varieties of bacteria introduced with the bean develop at sharply defined horizontal levels, which slowly extend toward the top. Certain varieties form several layers above one another. I have had these interesting statements verified by Mr. Miodowski, and have substantially confirmed them, with the exception that we found a bacterium related to the Bac. mesentericus and the Bac. subtilis predominantly present, instead of the non-sporulating Bac. perlibratus Beij., which Beijerinck found to principally compose the layers. (Compare Beijerinck, C. B. XIV, 827; C. B. L. III, 1; and Miodowski, Dissert. Würzburg, 1896.) In his second work especially, Beijerinck has related a number of interesting observations, but I am unable to enter into details regarding them, nor upon the analogous studies of Jegunow (C. B. L. II).

Schenk has observed a **positive thermotropism.** If a hanging drop is warmed at one point by a warm wire (temperature difference of 8° to 10°), the bacteria struggle toward it (C. B. XIV, 33).

2. OPTICAL ACTIVITY.

There are found, fairly widely distributed, especially in media rich in salt (sea-water, Elbe, salt-fish), **fission fungi which emit light,** of which a considerable number, mostly bacteria and vibriones, have been studied. The phosphorescence is a **life-symptom** of the bacteria and does not depend upon oxidation of a photogenic substance separated from the bacteria (K. B. Lehmann and Tollhausen, C. B. v, 785). Everything that interferes with the life of the bacteria, lessens it also; cold makes the organisms rigid, and interrupts the phosphorescence while it continues. High temperature, acids, chloroform, etc., disturb the light-phenomenon momentarily. Living cultures may always be obtained by inoculating from cultures that emit light. The germ-free filtrate never gives light. While no light is given off except when the bacteria are alive, still the live bacteria do not necessarily emit light; for example, in an atmosphere of CO_2. Similarly, a muscle can not contract except it is alive, but may be alive without contracting. (Compare also Suchsland, C. B. L. IV, 713.)

According to Beijerinck (C. B. VIII, 616 and 651), all light-giving bacteria, which he places in a (physiologic) "genus," **photobacterium,** require peptone and oxygen in order to emit light. Two of his varieties are satisfied with this; the four others require, besides peptone, also a source for carbon, which may also contain nitrogen. As such, small quantities of sugars (dextrose, levulose, galactose, partly maltose) and glycerin, as also asparagin, are suitable. A higher proportion of sugar, because of marked fermentation and production of acid, stops the emission of light in some cases. As for salts, 3% to 4% of sodium chlorid is favorable, magnesium chlorid appears to promote the production of light still more, while sea-salt is best.

To preserve the photogenic function it is best to employ a gelatin nutrient medium, which is prepared from an infusion of fish in sea-water (or artificial sea-water containing 3% of sea-salt) with the addition of 1% of peptone, 1% glycerin, and 0.5% asparagin. But even on this medium, if the transfer is infrequent, the ability to produce light is soon lost, so that the cultures found in laboratories are not usually photogenic. By repeated frequent transfer to suitable media the photogenic property may often be regained. I employ two salt herrings, cooked in a liter of water, and, after filtering, add 10% gelatin without neutralization.

3. THERMIC ACTIVITY.

The production of warmth during the metabolism of bacteria is absent from our ordinary cultures because of their limited size; even luxuriantly growing, fermenting fluid cultures betray no appreciable warmth to the hand. On the contrary, it is undoubtedly true that the heat exhibited by decomposing organic materials, when stored in a moist condition, as tobacco, hay, manure, etc., depends, at least in part, upon bacterial activity. With the high temperature which thus occurs, the conjecture of Lydia Rabinowitsch, that here the thermophilic bacteria are concerned, seems very probable. Accurate investigations into the causes of these high temperatures are still lacking. (Compare Rabinowitsch, Z. H. xx, 154.)

4. CHEMICAL ACTIVITY.

The chemical activities of bacteria, accompanied partially by the production of light and always by a minimum amount of heat, are to-day, in spite of the very numerous and satisfactory investigations of the last twenty-five years, only known in the roughest outlines. We often know only the principal products, without being accurately informed regarding the mechanism of their origin, the intermediate products, or the bodies occurring in small quantities.

The following **three principal varieties of chemical activity** may be distinguished:

1. The bacteria elaborate their own **body substance.** Regarding this the most important points have already been discussed in the proper place.

2. The bacteria secrete **ferments,** intended to make the nutrient medium in their neighborhood more suitable for assimilation. The products which thus occur in the surroundings of bacteria may be designated as metabolic products.

3. The bacteria assimilate materials and elaborate others which are true **metabolic products.** It is **wrong in principle** to make a division into **fermentation** and **metabolic products,** as is still sometimes attempted, be-

cause materials are only fermented after they are taken into the bacterial cell. **Fermentation products** are **metabolic products produced under the influence of special nutrition.** (Compare p. 64.)

I. The Bacterial Ferments and the Changes Produced by Them.

Under **ferments** in the restricted sense—**enzymes**—(the custom of calling micro-organisms "living ferments" is passing into disuse) one understands certain chemical bodies, which, in a minimal quantity and without being thereby destroyed, are able to split up large quantities of definite elaborate organic molecules into smaller, simpler, more soluble, and more diffusible ones.[1]

We can only properly speak of chemical ferments after the following properties have been demonstrated:

1. Fermentation continues in the presence of materials which are surely bactericidal, but do not injure ferments; for example, phenol, 3%; thymol, 0.1%; chloroform, ether; or
2. The power of producing fermentation is possessed by the germ-free filtrate, obtained by passing cultures of the bacteria through clay or porcelain cylinders; or
3. This activity is possessed by the pulverized and sterile ferment-preparation obtained from cultures.

Of the extraordinarily numerous details which have been taught by Fermi's[2] methodical and exhaustive studies, only the most important can here be given. All ferments dialyze a little, like ordinary albuminous bodies, through good parchment paper.

Proteolytic or **albumin-dissolving enzymes** are widely distributed. The liquefaction of the glue in gelatin (closely related to albumin chemically) is a sure indication of the presence of a proteolytic ferment. Since the reaction of the gelatin when dissolved is always or may be alkaline, the liquefaction is not due to pepsin (which is active only with an acid reaction), but to a *trypsin.* The individual bacterial trypsins differ very much as regards resistance to heat (they withstand 55° to 70° moist heat for one hour), susceptibility to injury by various acids, etc. Some are active with a suitable acid reaction, but never *more so* than with an *alkaline* reaction.

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[1] This definition evidently does not apply to rennet ferment, which coagulates milk.

[2] A. H. x, 1; xii, 240; C. B. xii, 713; C. B. L. i, 482.

Much more feeble than the action upon glue, is that upon fibrin.[1] Fermi has employed the following **method** as the easiest and surest way of proving the presence of even traces of proteolytic ferments: Tubes of the same size are filled to an equal height with an unneutralized 7% solution of gelatin in 1% aqueous solution of carbolic acid. The solution to be tested for proteolytic ferment has 2% carbolic acid added to it, and is placed in layers upon the solidified gelatin. The tubes are kept at room temperature and observations are made by means of a millimeter scale, as to how much the liquefaction of the gelatin extends in the course of days and weeks. For qualitative examination, the upper layer may consist of 1 c.c. of a liquefied gelatin culture, sterilized with carbolic acid.[2] This material also suffices if one wishes to test the influence of the nutrient medium on ferment formation. One may also, by this method, compare the action of various concentrations of different purely prepared bacterio-trypsins. The lower the percentage of gelatin and the nearer the temperature approaches that of the incubator, the more certainly will one observe effects from traces of ferment. In such critical cases the examination is continued fourteen days, and it is determined whether the gelatin remains fluid in the ice-box, while that in the control tube solidifies.

To *demonstrate the formation of true peptone* from the albuminous bodies one proceeds as follows:

The variety of bacterium to be tested is grown upon a fluid nutrient medium, rich in albumin, but containing no peptone (blood-serum, milk-serum, milk). After the culture is grown, all albuminous bodies except peptone are precipitated by the addition of strong ammonium sulphate (about 30 gm. to 20 c.c.). Milk and milk-serum may be warmed to 60° to 80° and blood-serum to about 40°. The precipitate is filtered off, and the filtrate cooled. A sample is made strongly alkaline with potassium hydroxid, and 1% solution of copper sulphate added drop by drop. A rose-red color indicates the presence of peptone.[3] Fermi has shown, by similar methods, that no variety of bacterium produces true peptone.

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[1] Fermi found only a few bacterio-trypsins acting upon fibrin, and none upon egg-albumin.

[2] Naturally, a control test with 2% carbolic-water (ferment-free) must never be omitted.

[3] Through more recent investigations it is certainly known that some albumoses besides peptone remain partly unprecipitated by ammonium sulphate.

The production of proteolytic ferments fluctuates with many, perhaps with all, species in a greater degree than one would be led to suppose from the ordinary descriptions. Beijerinck found that one of two photogenic vibriones at first liquefied slowly, but that after longer culture gelatin was always liquefied more rapidly; the other showed exactly the opposite. The same was observed by Katz in the Australian photogenic bacterium. Max Gruber and Firtsch (A. H. VIII, 369) have studied particularly closely liquefying cultures of Vibrio proteus, but they have also reported similar experiences with the cholera vibrio, Bact. vulgare, the Micrococcus pyogenes; indeed, many observers have even seen liquefying Streptococci pyogenes.

We have observed, also, in many varieties, that upon thin plates single, distinctly visible, superficial colonies of the same bacterium present such varying degrees of liquefaction that a beginner could scarcely be convinced that several varieties were not present.

It is very unfortunate that, through these observations, one of the readiest applied diagnostic aids, **the liquefaction of gelatin, has lost not a little in value.**

The causes of the decrease and increase of liquefaction under prolonged cultivation we ascribe to our artificial nutrient media or to the influence of the metabolic products of the micro-organisms, but without being able to give anything more decisive.

Regarding the **influence of nutrient media** upon the formation of trypsin in a culture and the **liquefaction** of gelatin, the following facts are known:

1. Most circumstances which interfere with the growth of a variety of bacterium on a nutrient medium also interfere with the liquefaction of gelatin; for example, the addition of phenol and a large amount of glycerin. Wood has observed that the lessened power of liquefying gelatin produced by phenol may be propagated for several generations upon favorable nutrient media (C. B. VIII, 266).

2. In hydrogen and nitrogen the liquefying facultative anaerobes do not liquefy gelatin,[1] while in CO_2, if they

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[1] A single exception is the B. prodigiosum; but if grape-sugar be added to the gelatin, it also ceases to liquefy.

can thrive in it, the contrary is true.[1] (Compare Table I.) Since the gases, according to Fermi, are without influence on the *activity of the ferment*, they must influence the *formation of ferment*. On the contrary, obligate anaerobes preponderately present most beautiful liquefaction of gelatin.

3. The addition of sugar does not interfere with the growth, but does with the liquefaction of gelatin in the case of many bacteria; for example, Bact. vulgare (Proteus vulgaris) (Kuhn, A. H. XIII, 40).

Auerbach has shown in my Institute (A. H. XXXI, 311) that sugar influences, in varying degree, the liquefaction of gelatin by various bacteria. In the instances examined, this checking was dependent upon the fact that no proteolytic ferment was formed in media containing sugar, and not that the sugar or acids formed from it interfered with the action of the ferment.

4. In fluid non-albuminous nutrient media, containing glycerin but no sugar, only a few bacteria produce proteolytic ferment; for example, B. prodigiosum and B. pyocyaneum. Also in peptone bouillon the ferment formation appears less than in peptone bouillon gelatin (Fermi).

Upon albuminous nutrient media the liquefying bacteria produce *bitter-tasting* metabolic products; for example, from milk in the case of many varieties (Hüppe).

An enumeration of the varieties forming trypsin may be omitted, since they are characterized by their **liquefaction of gelatin.** The other bacterial ferments have been less thoroughly studied.

Diastatic ferments change starch into sugar. They are recognized in this way: To a thin starch paste containing about 1% thymol, a culture containing 1% to 2% thymol is added. After keeping it in the incubator from six to eight hours, it is tested for sugar with Fehling's solution, when it is recognized by the reduction of the copper salt (reddish-yellow precipitate). One may also examine directly potato infusion cultures of bacteria for sugar, in which case the sugar is extracted by boiling with alcohol and the extract evaporated to a syrup. It is then dissolved in water and the reaction carried out.

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[1] It is questionable whether, in these experiments, equal care was always taken to absolutely exclude oxygen.

According to Fermi, about one-third of the varieties investigated possess the ability to form such ferment, but only on albuminous nutrient media (A. H. XI, 1, and C. B. XII, 713). It is produced by bacilli of the subtilis group (anthrax, megatherium, Fitzianus, etc.), the vibriones related to the cholera vibrio; besides, Micrococcus tetragenus, Micrococcus mastitidis, Bact. janthinum, Bact. mallei, Bact. pyogenes fœtidum, Bact. phosphorescens, Bact. pneumoniæ, Bact. synxanthum, Bact. aceticum. The remainder do not elaborate it or it is doubtful whether they do. Besides, all actinomyces and Oosporeæ (with the exception of the Oo. carnea) form such a ferment. Most of these varieties afterward utilize the sugar further to form acid, while others do not; for example, Bacillus subtilis.

Inverting ferments—*i. e.*, such as convert cane-sugar into grape-sugar—are rare, according to Fermi and Montesano (C. B. L. I, 482). Their presence is easily demonstrated by mixing a 1% to 2% solution of cane-sugar containing carbolic acid and the culture treated with 1% carbolic acid, and after a few hours testing with Fehling's solution, and learning if it reduces the solution after standing, which cane-sugar admittedly does not. Control experiments with a solution of cane-sugar alone are always necessary. Fission fungi invertin can (always?) stand 100° for over an hour; it is also produced in non-albuminous nutrient media, if glycerin is added. As producers of inverting ferments the above authors mention only: Bacillus megatherium, B. kiliense, B. fluorescens liquefaciens, B. vulgare, and Vibrio choleræ and Metschnikovii.

Efforts to find a ferment resembling **emulsin** have been frustrated. The "Micrococcus pyogenes tenuis" splits off benzaldehyd from amygdalin, but without the function being separated from cell-life.

Rennet ferments—*i. e.*, bodies which coagulate milk of neutral or amphoteric reaction, unconnected with the action of acid—are not lacking among the products of bacteria. For example, cultures of the Bact. prodigiosum, if not too old, sterilized by heat at 55° to 60°, cause a solid coagulation of sterile milk in one or a few days (Gorini, C. B. XII, 666).

Thorough investigations regarding the distribution of this ferment are unknown to me. We may suspect it in all varieties which coagulate milk without being able to form lactic acid from milk-sugar.

II. The Chemical Activity of Bacterial Metabolism.

As with ferment production, so also most other **chemical activities** of bacteria depend in great measure upon **nutrient media.** This is most striking when one observes the growth of several varieties of bacteria upon albuminous nutrient media, at one time **without sugar** and again **containing sugar.** While in the first case, except for the formation of pigments and some odoriferous substances, there is scarcely any appreciable metabolism, in the second case there often occur striking distinguishing changes characterized by the development of gas and active production of acid. Thus the organism produces "fermentation" in the medium containing sugar; in the other, none.

Because of the practical (and diagnostic) importance of the ability to produce fermentation, there must first be given a precise definition of the process.

The expression **"fermentation"** is employed in the literature with the most varying meanings.

1. Many authors call every typical decomposition caused by bacteria "fermentation," and speak, for example, of the putrid fermentation of albuminous bodies.

2. Others limit the word "fermentation" to processes which are accompanied by evident gas bubbles. According to this definition, the liberation of nitrogen from saltpeter is as much fermentation as the breaking up of milk-sugar by the Bact. acidi lactici.

3. Still others only speak of fermentation when there occurs a breaking up of carbohydrates, with or without gas production.

To me the expression **"fermentation"** *is only applicable when it is proved that an organism produces, along with or instead of its other metabolic products, one or more special metabolic product in striking amount; products of metabolism which almost always arise from the merely superficial splitting up of an easily decomposed bacterial nutrient material (splitting fermentation).* Oxidation fermentation is more rare (compare below). *The* **essential for fermentation** *is always the* **presence** *of a special* **nutrient material,** *which the fungus appropriates very easily, often rejecting materials more* *difficult of utilization, which it would reduce in the absence of fermentable objects.*

Every fermentation has the object of furnishing a **store of energy** to the fermenting organism. This is attained in the **splitting fermentation** in this way:

In the interior of the bacterial cell the complicated fermentable molecule is decomposed into smaller fragments, and thus energy is set free. I will illustrate this as it occurs in the common fermentations of sugar, where the case is very simple:

$$C_6H_{12}O_6 = 2C_2H_6O + 2CO_2$$

1 Grape-sugar = 2 Alcohol + 2 Carbon dioxid.

Or

$$C_6H_{12}O_6 = 2C_3H_6O_3$$

1 Grape-sugar = 2 Lactic acid.

Or

$$C_6H_{12}O_6 = 3C_2H_4O_2$$

1 Grape-sugar = 3 Acetic acid.

Organisms growing with oxygen excluded especially utilize one of these sources of energy, as the source of energy at the command of aerobic varieties, residing in the oxidation of resorbed substances through absorbed oxygen, is cut off. Therefore all anaerobic varieties are endowed with the ability to cause active fermentation of sugar, while many facultative anaerobes only cause fermentation of nutrient media containing sugar when oxygen is excluded.

As already mentioned on page 29, Buchner has discovered that by expression under great weight a ferment (zymase) can be obtained from the bodies of yeast-cells which ferments sugar most intensely. These discoveries have advanced considerably our understanding of the utilization of sugar within the cells of the yeast fungus. We now know that the breaking up of sugar and the resulting gain of power originates simply "through the vital process," but we recognize, in the zymase, the special means which the organism makes use of for this activity. Heretofore it has not been possible to obtain zymase or other intracellular acting ferments from bacteria, yet, for the present, we may suppose that yeasts and bacteria are similar in this respect.

A counterpart to splitting fermentation is the rarer **oxidation fermentation,** of which the most beautiful example is the formation of acetic acid from alcohol. Here, likewise, there occurs one-sided metabolic activity by the acetic-acid fungus, which obtains a large supply of energy, not through the splitting up of an easily decomposed substance, but through oxidation of resorbed alcohol. The gain of energy is here dependent simply upon a partial development and enhancement of the ordinary phenomena in the nourishment of bacteria.[1]

According to this, fermentation products as well as all other products of bacterial cells are metabolic products, and a special separate treatment of fermentation is not demanded. On the contrary, it seems most suitable to arrange the discussion of the single bacterial products acording to whether they originate in nutrient media which contain sugar, or are free from it, and to add something concerning such activities of bacteria as result in the breaking up of salts of fatty acids, alcohols, etc.

1. Pigment Production.

The pigments have been but little studied chemically, yet recently, through several pupils of Migula, at least a provisional survey has become possible. Compare Schneider (A. K. I, 201) and Thumm (A. K. I, 291).

The **red** and **yellow** pigments which have been thoroughly studied are almost all[2] insoluble in water, but soluble in alcohol, ether, carbon bisulphid, benzol, and chloroform. For the present they may be placed in two groups:

(*a*) Pigments of the **carotin** group. Yellow, orange, rose colored, with strong sulphuric acid becoming bluish-green ; with caustic alkalis, orange to red. In some cases the pigments, which often consist of a mixture of several pigments, present great variation. (See Schneider, A. K. I, 201, regarding spectra and peculiarities.) They are, however, closely related to the widely distributed lipochromes (pigment substances of fats, yolk of egg, etc.) and the carotin of yellow carrots. (Compare Leisenberg and Zopf, C. B. XII, 659.)

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[1] Certainly splitting fermentation is always a restricted, and only under certain circumstances a highly developed, function.

[2] In marked contrast to this are the findings of M. Freund (C. B. XVI, 640), according to which the red and yellow pigments produced by four newly discovered bacteria were always soluble in water and insoluble in alcohol and ether.

(*b*) **Prodigiosin** pigments. By prodigiosin I designate the beautiful pigment of the Bact. prodigiosum and its nearest relatives. It is soluble in ether as yellowish-brown and in alcohol as garnet-red. It is turned yellow by alkalis, violet-red by acids, and brownish-red by concentrated sulphuric acid. Zinc and hydrochloric acid reduce the pigment to a colorless leuko-product. The spectroscopic behavior is very characteristic.

Violet pigments. In connection with the bacterium violaceum, and also the Bacterium janthinum, there is produced, according to Schneider (verified by myself), a violet pigment (**janthin**) which is insoluble in water, readily soluble in alcohol, but insoluble in ether, benzol, and chloroform. If dry, it becomes yellow when treated with concentrated sulphuric acid and emerald-green when treated with caustic potash. In alcoholic solution all strong acids and ammonia produce a green or bluish-green color. With zinc and sulphuric acid the color is destroyed (Schneider, *l. c.*).

The beautiful blue pigment of the Bact. indigonaceum Claessen was very incompletely examined by Claessen and Schneider (*l. c.*). This pigment is not dissolved by ordinary solvents. Hydrochloric acid gives a transitory blue, turning to a yellowish-brown solution. Also, other acids in dissolving it cause its decomposition. Caustic potash turns the color bluish-green. I am unable to add anything further.

Different from these is the **blue** pigment produced by the Bacterium syncyaneum (blue milk), which I propose to call **syncyanin.** It is also entirely independent of the bacterio-fluorescein forms (see below). This pigment was pointed out by Thumm as very unstable; acids turn it steel-blue, in weaker acids it is blue-black, neutral it is black, alkaline it is brownish-black. For details see the special part.

The **fluorescent pigments** which occur in the cultures of very many bacteria are identical, according to recent investigations by K. Thumm. The pigment which I propose to call **bacterio-fluorescein**, when dry, is lemon-yellow and amorphous. It is soluble in water and dilute alcohol, insoluble in strong alcohol, ether, and carbon bisulphid. The aqueous solution, when concentrated, is orange; when diluted, pale yellow. The solution, when acid in reaction, presents no fluorescence; when neutral, a blue; and when alkaline, a green fluorescence. In the culture the fluorescence is at first blue, and later, because of the ammonia produced by the bacteria, becomes green. The pigment is not sensitive to oxidizing agents. Colorless antecedents (leuko-bodies) are not observed. Phosphoric acid and magnesium appear to be essential for the production of bacterio-fluorescein. (See also E. O. Jordan, Botanical Gazette, XXVII, 19.—ED.)

We have more exact knowledge concerning the beautiful blue crystalline pigment, **pyocyanin** ($C_{14}H_{14}N_2O$). It can be easily extracted from cultures of the Bact. pyocyaneum with chloroform, and separated from the bacterio-fluorescein. Thumm has entirely overlooked it.

Black-growing varieties of bacteria have been but little studied. According to Marpmann (C. B. L. IV, 21), the black color is usually (always) dependent upon a granular secretion of sulphid of iron. It is thus easily understood why the "pigment-production" stops upon transferring to nutrient media free of iron. The almost black forms of the Bact. cœruleum are, however, certainly not colored by sulphid of iron (Lehmann).

There have been many investigations regarding **fluctuation of the chromogenic function.** All possible influences which affect the growth of the bacteria unfavorably also lessen the production of pigment. After continued cultivation upon unfavorable media or at unfavorable temperature, etc., the chromogenic function of the descendants may remain permanently reduced. Thus, there occur, for instance, examples of the Bact. syncyaneum which no longer produce a trace of pigment in agar and milk (compare Behr, C. B. VIII, 485), but which color potato darkly in the neighborhood of the culture.

Pigment production appears to be lessened by merely infrequent transfers of the agar culture.

The Bact. prodigiosum at 37° forms no pigment; if grown at this temperature for a long time with constant transfers, the power of pigment formation is, even under favorable circumstances, lost for many generations (Schottelius).

Very interesting experiences with **pigment-forming cultures** of varieties which usually produce **colorless growths** are scattered through the literature; for example, Fawitzky regarding yellow to rusty-red colonies of Streptococcus lanceolatus; Kruse and Pasquale on colored varieties of the Streptococcus pyogenes (Ziegler's Beiträge, XII); also the experience recently published by Kutscher, according to which a pseudoglanders bacillus, when obtained from the animal, in the primary culture upon serum develops a bright orange-red growth, but after a few transfers the red color is completely exchanged for a white one (Z. H. XXI, 156). Perhaps still more important is the observation, which is easily made, that, from some internal condition, at times colored and colorless colonies of the same variety grow side by side upon a plate; for example, in Bact. kiliense. R. O. Neumann has, by selection, grown from the Micr. pyogenes α aureus white, yellow, and red varieties (A. H. XXX, 1).

An analogue to this variation from internal causes is related by F. Hildebrand, who observed in a stock of Iris florentina, which always bore pale-blue flowers each year, the sudden appearance of two flowers presenting dark violet portions arranged in sectors (Ber. d. deutsch. bot. Gesell., 1873, 476).

2. The Formation of Ammonia and Urea-fermentation.

According to Sommaruga (Z. H. XII, 273), aerobic bacteria growing in **non-saccharine** nutrient media always form an **alkali** from albuminous bodies.

When **sugar is present,** most varieties, besides producing alkalis, form **acids** from the sugar. In this way is explained the fact that many young cultures of bacteria at the beginning are neutral or faintly acid in reaction because of the small amount of sugar in the bouillon (originating in the meat). When the sugar is exhausted, then alkali production advances more strongly (Th. Smith).

The bodies causing the alkaline reaction, so far as known, are ammonia (at times it may be smelt), amine, and ammonium bases. To determine the amount of alkali formed, one titrates single tubes which contain 10 c.c. of peptone bouillon, both uninoculated and one to fourteen days after inoculation, with decinormal acid, using phenolphthalein as indicator. The difference upon titration gives the increase of alkali.

The following may be useful as an example of the production of alkali by bacteria which, in the presence of sugar, form acids energetically (for 100 c.c. amounting to 5 c.c. to 7 c.c. normal acid). One hundred c.c. of a nutrient medium was employed, which contained a trace of meat-sugar, and was originally exactly neutral with phenolphthalein.

	AFTER FIVE DAYS.	AFTER TEN DAYS.	AFTER FIFTEEN DAYS.
Inoculated with Bacterium coli.	0.1 normal alkali.	0.1 normal alkali.	0.25 normal acid.

A special instance of the production of an alkali by bacteria occurs in the transformation of urea into ammonium carbonate, $CO(NH_2)_2 + 2HO_2 = CO_3(NH_4)_2$.

In 1896 we stated that of sixty varieties tested, only the Bact. vulgare, Bact. prodigiosum, and Bact. kiliense were found able to decompose urea. Brodmeier (C. B. XVIII, p. 380) has investigated the urea-splitting property quantitatively with the Bact. vulgare, and my pupil, Dr. Mann, has recently done the same with the Micrococcus pyogenes α aureus and γ albus, with two forms of the colon bacillus, and several sarcinæ. One hundred c.c. of filtered urine, sterilized at 85°, after being ten days in the incubator contained abundant NH_3. Mann could not demonstrate any action by the same culture of the Bact. prodigiosum, which we had previously found to cause energetic fermentation of urea. Thus, this property also is variable, and the contradictory results of authors with the Bact. coli (see special part) and the Micr. pyogenes are thus explained.

What are described in the literature as Micrococcus ureæ Leube, Bacillus ureæ Leube, Bacillus ureæ liquefaciens Flügge, can be partially identified as the Micr. pyogenes γ albus and Bacterium coli, but the descriptions of these varieties allow of no accurate identification. The urea-splitting function appears to occur occasionally in very many varieties. Warington (C. B. VI, 498), Burri, Herfeldt and Stutzer (C. B. L. I, 284) have described urea-splitting varieties. Compare also the investigations of Miquel (Ann. d. Micrographie, Bd. I u. f.), which are very interesting biologically, but which lose much in value because Miquel has elaborated a very singular nomenclature which does not take into consideration the usual varieties. Miquel has observed varieties which are able to decompose as much as 60 gm. of urea to a liter. He claims to have isolated a special ferment, **urase,** which decomposes urea.

The older literature can be found as given by Leube (Virchow's Archiv, Bd. C, p. 540); the newer, with the method of determining ammonia (according to Schlösing), by Mann.

3. Formation of Complicated Basic Metabolic Products.

Especially through the investigations of Brieger (Ueber Ptomaïne, Heft I–III, Berlin, Hirschwald), besides ammonia, a large number of basic, crystalline, nitrogenous bodies are known as products of bacterial metabolism. These bodies are usually called ptomains (*πτῶμα*, putrefaction) or **putrefaction alkaloids.**[1] They occur, so far as closely studied, mostly in the following groups:

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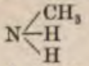
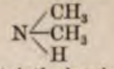
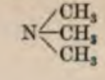
[1] For a long time the *poisonous* ptomains were called *toxins*, yet now most authors call **all bacterial poisons toxins**, without reference to their chemical constitution, and usually one understands the term to include the "albuminous-like" bacterial poisons more especially.

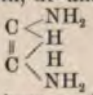
1. **Amins.** Methylamin, di- and trimethylamin :







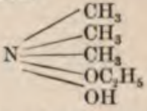
similarly, ethylamin, di- and triethylamin.



Ethylendiamin and its homologues dimethylethylendiamin-**putrescin,** isomer of **sepsin ;** pentamethylendiamin, called **cadaverin,** etc. Of these the most poisonous is ethylendiamin.

2. Ammonium bases. The best known is

Cholin = **Bilineurin** =



Nearly related are **muscarin** $(C_5H_{15}NO_3)$, **vinylcholin** $(C_5H_{13}NO)$, **neuridin** $(C_5H_{14}N_2)$, etc.

3. **Pyridin derivatives.** Derived from **pyridin** (C_6H_5N), the following are especially found : **Collidin** $(C_8H_{11}N)$, **parvolin** $(C_9H_{13}N)$.

4. **Indol** (C_8H_7N) and **skatol** (C_9H_9N). Compare page 79.

In addition the following are known : **Amido-acids** (leucin, tyrosin, etc.) related to **guanidin,** $C(NH)(NH_2)_2$, and also numerous, insufficiently or feebly characteristic bodies, whose enumeration here would be useless, since the poisons among them are not now recognized as the **essential disease poisons,** as was the case in former years.

The *isolation* of these bodies can here be only hinted at. The method of Brieger, which is most employed, is as follows : Brief boiling of the culture or "decomposed material" rendered weakly acid with hydrochloric acid ; reduction of the filtrate to a syrup ; dissolve in 96% alcohol and free from impurities (especially traces of albumin) with lead acetate ; removal of the lead ; concentration of the filtrate and precipitation from this of the double salt of mercury of the ptomain by means of an alcoholic sublimate solution. After removal of the alcohol by heat and the mercury by hydrogen sulphid, there is produced the characteristic double gold and platinum compound, whose crystalline quality is an index of its purity. One may try to obtain directly the crystalline hydrochlorate, and by the aid of caustic soda the free bases, which are often fluid.

Some ptomains, like very many plant alkaloids, as soon as set free by caustic soda, are easily obtained in aqueous solution with ether. Still, Brieger's procedure is widely useful, as it takes into consideration many bodies which do not dissolve in ether. For extensive review of literature upon ptomains, see Jacquemart (C. B. IX, 107).

4. Production of Complex "Albumin-like" Poisonous Metabolic Products ("Toxalbumins," Toxins).

In addition to the discussion of the relatively simply formed, basic, less poisonous metabolic products of bacteria, we may speak, as briefly as possible, of the other **bacterial poisons.** From the standpoint of our present knowledge, they may be divided into three classes:

1. **Bacterioplasmin** (Buchner). Compare Hahn, Münch. med. Wochenschr., 1897, No. 48, 1344. The expressed juice (compare pp. 29 and 65) of bacteria contains, in ordinary unchanged form, poisonous substances; present in the bacteria of cholera, typhoid, and tuberculosis; absent in Micr. pyogenes and Bact. anthrax. Koch's new tuberculin, "T R," is essentially also a plasmin.

2. **Bacterioprotein** (Buchner). Under this head are placed albuminous bodies, **unaltered by heat,** which produce fever (pyrogenic), inflammation, and suppuration[1] (phlogogenic). They are obtained by boiling for several hours scraped potato-cultures with 0.5% caustic potash (about 50 volumes KHO to 1 volume of bacterial substance). The protein may be precipitated from the clear fluid obtained by filtering through paper, by carefully rendering it feebly acid. The protein is filtered out, washed and dried, and before being employed is dissolved in a small quantity of weak soda solution.

The best-known protein is the "old" **tuberculin** of Koch; also **mallein** belongs here. According to Buchner and Römer, all bacterial proteins act alike and not specifically.

3. **"Toxalbumins,"** now usually called **toxins.** The isolated statements of earlier investigators (Christmas, Roux and Yersin, Hankin) were confirmed to a great extent by the investigations of C. Fränkel and Brieger (Berl. klin. Wochenschr., 1890, 241 and 268), who found that

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[1] Suppuration is best produced by bacterial and non-bacterial products if they slowly diffuse from a gelatin capsule into the subcutaneous tissues (Poliakoff, C. B. XVIII, p. 33).

agents which precipitate albumin are able to precipitate, from the bouillon culture of many bacteria, amorphous poisons, which possess intense and, indeed, almost always specific action (like the living cultures). They call these poisons *toxalbumins*, and compare them with the "poisonous albuminous bodies" obtained from many plants (ricin from Ricinus communis, abrin from Abrus precatorius, etc.). Most investigators have regarded, and some still to-day regard, the poisons as **unstable albuminous bodies,** which have their origin in the bacterial cells. Often, also, they are compared to snake poisons and enzymes. Like these bodies, they possess a **great sensitiveness to heat, reagents, light,** etc.

The **toxin** may be **obtained** as a crude product by precipitating it with absolute alcohol or ammonium sulphate from an old bouillon culture of the bacteria, which had previously been freed from living organisms by a porcelain filter,[1] and concentrated in vacuum. If ammonium sulphate has been used, it must first be removed from the precipitate obtained upon filtering by dialysis with flowing water in a parchment coil, and then the toxin precipitated with absolute alcohol after renewed concentration in vacuum. Recently we have learned that zinc chlorid precipitates the bodies quantitatively, and from the precipitate the toxins can be obtained by precipitation of the zinc with ammonium bicarbonate and ammonium phosphate. In the filtrate the toxin is precipitated with ammonium sulphate. For detailed communications upon this, see Brieger and Boer (Z. H. xxi, 259, and Deut. med. Wochenschr., 1896, No. 49, 783).

From the first there were strong misgivings that these "toxalbumins" were only bodies carried down by precipitated albumins, but having nothing to do with albumins proper.

In connection with tetanus poison it has been possible for Brieger and Cohn (Z. H. xv, 1) to obtain from the crude poison, under great precautionary measures, by means of lead acetate and ammonia, a pure poison. This presents with copper sulphate and caustic soda a faint violet color, but otherwise *no reaction for albumin.* It is free from phosphorus and almost entirely free from sulphur. Thus it seems to be demonstrated that the **tetanus poison is not an albuminous body.**

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[1] Many authors have recently proved that toxins are held back in part by thic[illegible] filters.

Also the **cholera** and **diphtheria poisons** are to-day recognized by Brieger and his pupils as non-albuminous, or at least not as "albuminous bodies" in the ordinary sense. The statement of Uschinsky, also, that at least certain diphtheria cultures form toxins in non-albuminous nutrient media, is not to be questioned. But in contrast to the bacterial toxins the powerful plant-poisons, abrin and ricin, which in their properties present many similarities to the former, are said to be true albuminous bodies.

Regarding the other properties of these toxins, I will give some of the recent statements, using the tetanus poison as an example (Brieger and Cohn, *l. c.*). The toxin does not diffuse through membrane, and consequently is purified by dialysis (Fedoroff, C. B. xvi, 484). Watery solutions are not coagulated by heat, but in time lose their poisonous properties. The poisonous property is very much injured by the addition of small amounts of acids or alkalis to the solution and by the prolonged passage through it of carbonic acid and sulphuretted hydrogen. When dry, the toxin withstands 70° for a long time, while higher temperatures destroy it rapidly. In a dry condition, protected from light, air, and moisture, it is slowly converted into an inert body. It keeps better if covered with absolute alcohol, anhydrous ether, and the like.

The statement is interesting that very small quantities of bile, pancreatic secretion, etc., suffice to destroy large quantities of diphtheria and tetanus poison (Nencki and Sieber, C. B. xxiii, 880). According to Ransom, tetanus poison, introduced by mouth, remains unabsorbed and escapes from the body by the rectum (Deut. med. Wochenschr., 1898, No. 8, 117).

The **toxicity** of the purest tetanus poison obtainable is **almost unbelievable.** A mouse weighing 15 gm. is killed by 0.00005 mg.; a man weighing 70 kilos, if equally susceptible, would be killed by 0.23 mg. Of strychnia, 30 to 100 mg. are required to cause death in man.

According to Courmont and Doyon, the toxins are not ready-formed poisons, but the body, during the time of incubation, furnishes the final poison through the development of a ferment. The blood and juice of muscles from animals suffering from tetanus act more promptly than the toxin. Similar results were obtained by Blumenthal (Deut. med. Wochenschr., 1898, No. 12, 185). G. G. Brunner contests this upon experimental grounds (C. B. XXIV, 184).

5. Sulphuretted Hydrogen.

Sulphuretted hydrogen is a **widely distributed** bacterial product. It may be simply demonstrated as follows: By means of the cotton stopper a strip of moist lead-acetate paper is fastened in the neck of the culture-tube, which is then closed with a rubber cap (made from dark rubber, free from sulphur). Frequent observations are necessary, as the paper, which at first is brownish and later blackish, and often only faintly discolored, still later fades out. One must not arrive at negative conclusions too soon. As the most beautiful method for demonstrating sulphuretted hydrogen, Ernst employed gelatin colored Madeira-yellow with sodium ferri-tartrate (ferrum tartar. oxydat. [Merk] 0.5, aq. 50.0, and Na_2CO_3 added until reaction is alkaline). This is turned black by H_2S. Literature: Petri and Maassen (A. G. A. VIII, 318 and 490) and Rubner, Stagnitta-Balistreri and Niemann (A. H. XVI, 53).

The sulphuretted hydrogen may be formed—

1. **From albuminous bodies.** (As is well known, *boiling* separates H_2S from egg-albumin.) This ability, according to Petri and Maassen, appears especially in fluid nutrient media rich in peptone (5% to 10%) and free from sugar, in connection with all the varieties studied, but in most variable degrees. In bouillon free from peptone only a few varieties (for example, Bact. vulgare) form H_2S, and in 1% peptone bouillon about 50% (Stagnitta-Balistreri). Among sixty varieties studied upon 2% peptone bouillon we found twenty-eight—*i. e.*, 47%—to be producers of H_2S. (See Table I, at end of book.)

2. **From sulphur powder.** All bacteria in nutrient media to which pure sulphur powder is added form really larger amounts of H_2S than without such an addition. Petri and Maassen point to this production of H_2S as a result of the action of nascent hydrogen, pro-

duced by the bacteria; that is, they look upon this production of H_2S as a demonstration of the formation of nascent hydrogen.

3. **From hyposulphites and sulphites.** Especially studied in yeasts, but also demonstrated in some bacteria by Petri and Maassen.

4. **From sulphates.** Beijerinck (C. B. L., Bd. I, 1) has proved this practically important function for his motile obligate anaerobic Spirillum desulfuricans, which has only slight morphologic characteristics. With other bacteria it is rarely found developed.

Rubner has pointed out that with the Bact. vulgare the liberated organic sulphur always suffices for the formation of H_2S.

The *presence of sugar* in nutrient media only rarely diminishes or prevents the formation of H_2S, even if the bacteria are able to actively ferment sugar. The breaking up of the carbohydrates does not protect the albuminous bodies from decomposition. The presence of saltpeter operates injuriously; under these circumstances only a little H_2S is formed, but abundant nitrite (Petri and Maassen). Exclusion of oxygen favors the formation of H_2S. With the passage of air through cultures of facultative anaerobes which produce H_2S, the amount of H_2S formed is markedly reduced, and, instead, sulphates are produced.

Some (probably many) bacteria which form H_2S also produce foul-smelling **mercaptan,** $CH_3—SH$, which is demonstrable by the green color that it imparts to the yellowish-red isatin-sulphuric acid. One places upon the culture-glass a tube, open at both ends, filled with glass pearls, which are moistened with a 1.5% solution of isatin in concentrated sulphuric acid. The presence of sugar in the nutrient medium lessens or prevents the production of mercaptan (Rubner, A. H. XIX, 136).

6. Reduction Processes.

(Reduction of pigments, nitrates, etc.)

We have seen that generally the aerobic bacteria are able to change powdered sulphur into H_2S, for which nascent hydrogen is necessary.

The following processes are, indeed, similar to, and in part probably dependent upon, nascent hydrogen:

1. **Reduction** of the complex **blue litmus-pigment** of **methylene-blue** and **indigo** to colorless leuko-products. The part near the surface in contact with the air often shows no reduction, but only the deeper layer. By shaking with air the color may return, but where there has been simultaneous production of acid, the returning color

is red. The method of procedure is self-evident; nutrient bouillon is the medium employed. According to Cahen, all liquefying bacteria reduce litmus. It may be observed very beautifully with, for example, the Bacillus fluorescens liquefaciens. There are also non-liquefying varieties, as, for example, Bact. coli, which present this characteristic.

2. **Reduction** of **nitrates** to **nitrites** and **ammonia.** The first property seems to be possessed by bacteria very widely, at least Petri and Maassen found pronounced nitrite production almost without exception in six varieties grown upon bouillon containing 2.5% to 5% peptone and 0.5% saltpeter; only once was ammonia alone found. Rubner (A. H. xvi, 53) failed to find nitrite production in isolated instances only; Warington found eighteen producers of nitrite out of twenty-five varieties. According to our observations, the addition of sugar did not interfere with the process in the case of the Bact. coli, typhi, vulgare, Bac. anthracis, subtilis, and Vibrio choleræ. After five days upon 0.5% saltpeter, 1% peptone-bouillon, the nitrite reaction was equally great with and without the addition of 1% of grape-sugar.

The **demonstration of nitrite** is conducted as follows: There is added to the nitrate bouillon—also to two uninoculated control tubes—after the tubes have been kept some days in the incubator, some colorless iodid of potassium-starch solution (thin starch paste with 0.5% iodid of potassium) and a few drops of dilute sulphuric acid. The control tubes remain colorless, or, at most, gradually become faintly blue. If, however, nitrite is present, there occurs a deep blue to (with an abundant amount of nitrite) a dark brownish-red color. Small amounts of nitrite are demonstrated with metaphenylendiamin and dilute sulphuric acid (yellowish-brown color) or (most distinctly) with a mixture of sulphanilic acid and naphthylamin (red color). Compare Dieudonné (A. G. A. xi, 508).

The **demonstration of ammonia** by the addition of **Nessler's reagent** is only allowable with inorganic and sugar-free nutrient media. In bouillon, almost immediately there occurs a reduction of Nessler's reagent to black mercurous oxid. Strips of paper wet with the reagent may be suspended above bouillon cultures, or the latter may be distilled after adding MgO and the distillate treated with Nessler's reagent. Yellow to reddish-brown color indicates ammonia. Control experiments must always be made.

7. Aromatic Metabolic Products.

Often, as the result of the action of very many varieties of bacteria, there arise from albumin aromatic bodies, of which **indol, skatol, phenol,** and **tyrosin** are best known. Methodical investigations are at hand regarding the occurrence of only indol and phenol, since these bodies are easily recognized.

Demonstration of Indol.—There is added to the bouillon culture, which is preferably not less than eight days old and prepared without the addition of sugar, about one-half its volume of 10% sulphuric acid. If, now, on warming to about 80° a rose or bluish-red color at once appears, then both *indol* and *nitrite* are present. The nitroso-indol reaction just described requires both these bodies to be present for its success. With cholera and most other vibriones, at times also with diphtheria, the demonstration may be made (**"cholera-red reaction"**). *Usually the addition of sulphuric acid is* **not** *sufficient*, and it is necessary to add also a **little nitrite.** This may be added if, upon warming without the nitrite, no reaction, or only a doubtful one, is obtained. One adds 1 c.c. to 2 c.c. of a 0.05% solution of sodium nitrite until the maximum reaction is obtained. Addition of a stronger nitrite solution colors the fluid brownish-yellow, and entirely prevents the demonstration of indol.

Demonstration of Phenol.—The culture in non-saccharine bouillon receives the addition of about one-fifth its volume of hydrochloric acid and is then distilled. The distillate gives a flocculent precipitate when treated with bromin water. If carefully neutralized with calcium carbonate, the addition of neutral very dilute chlorid of iron gives a violet color.

In sixty varieties examined, we found (see Table I) indol production twenty-three times. Our findings accord well with the statements of Levandowsky (Deutsch. med.

[illegible] The following are pro- [illegible] as a whole, glanders, [illegible] With the excep- [illegible] Levandowsky, those [illegible] also produce phenol. [illegible] only in Bacte- [illegible] found only traces of [illegible]

[illegible] Decomposition of Fats.

[illegible] medium for bacteria. [illegible] a pure chem- [illegible] action of oxygen [illegible] (2) a [illegible] milk-sugar pres- [illegible] xv. 354). [illegible] with produc- [illegible] as a nutrient me- [illegible]

[illegible] Putrefaction [illegible] Supplement to 1 to 7.)

Putrefaction [illegible] decomposi- [illegible] accompanied by **the formation of foul-smelling substances.**

[illegible] as [illegible] and [illegible] are the sub- [illegible] first peptonized and [illegible]

Typical putrefaction [illegible] occurs only with a deficient or limited supply of oxygen. Active passage of air through a putrefying culture of bacteria (an occurrence which never takes place in natural putrefaction) weakens the putrefactive power most actively. This is accomplished biologically, by killing or checking the [illegible] the anaerobic putrefactive bacteria, and also by the influence of oxygen upon the products or intermediate products of the aerobic and facultative anaerobic bacteria. Finally, it is probable that the same bacteria from the very start produce different putrefactive products when grown anaerobically from those produced under aerobic conditions.

As products of putrefaction we find those given in the preceding sections:[1] Albumoses, ammonia and amine, leucin, tyrosin and other amido-bodies, oxyfatty acids, indol, skatol, phenol; then, sulphuretted hydrogen, mercaptan, carbonic acid, hydrogen, and finally marsh-gas.

In the decomposition of different nutrient media by various fungi, the metabolic products just enumerated are found, as a rule, only in part and in most variable combinations, so that **putrefaction can scarcely be defined more exactly by chemical aids** than is possible by the **senses.** I am, therefore, of the opinion that it is best to employ the expression "putrefaction" only in the general sense of the laity to indicate every foul-smelling decomposition of albuminous bodies. (Compare Kuhn, A. H. XIII, 40.)

10. Nitrification.

According to Heraeus (Z. H. I, 193), the ability to form nitrite, at least in traces, from NH is widely distributed.[2] Most investigators, however, agree in stating that nitrite production from NH_3 exclusively, or at least preponderantly, is dependent upon an organism, possessing slight morphologic characteristics, which Winogradsky, in his original work, designated *nitrosomonas.* For more detailed description see special part.

Winogradsky has described somewhat more completely the organism which forms nitrates from nitrites, and which he calls "nitrobacter" (compare special part). Both organisms are alike in that they grow only upon poor nutrient materials—on mixtures of inorganic salts or agar and mixtures of salts without peptone or sugar—and do not grow on any of our ordinary nutrient media. The contradictory statements of Stutzer and his pupils are generally considered incorrect. Both organisms are widely distributed in the soil, in meadows often the nitrite producers alone, in cultivated soil usually both. Both organisms possess the greatest theoretic interest, since out of inorganic nitrogen and carbonic acid (both free CO_2 and Na_2CO_3 or the presence of a bicarbonate is necessary) they are able to build up their body substances, *i. e.*, albumin, without the aid of chlorophyll, which the higher plants require.

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[1] The assertion is often made that the albuminous bodies are first peptonized in every putrefaction, but since Bact. vulgare, B. Zenkeri, and Bact. putidum are generally recognized as "causes of putrefaction," but never liquefy gelatin, it is not proper to speak of peptonization of albumin as being always present in putrefaction.

[2] Rullmann calls attention to the nitrite present in laboratory air, which may easily cause mistakes (C. B. L. V, 212).

P. F. Richter (C. B. XVIII, p. 129) often observed marked nitrite reaction in urine freshly obtained with a catheter. From one specimen of urine he isolated a medium-sized coccus, which produced very intense nitrite reaction in fresh urine in twenty minutes. It also reduced nitrate to nitrite.

II. Transformation of Nitrites (and Nitrates) into Free Nitrogen (Denitrification).

An entire series of organisms, which are widely distributed in dung, straw, field-soil, and filthy water, set free gaseous nitrogen from nitrites (denitrification). Many are able simultaneously to transform nitrate into nitrite, also alone to set free gaseous nitrogen from nitrates (for example, Bacterium Stutzeri, B. pyocyaneum—compare special part), while others require synergetic bacteria to change the nitrate into nitrite (for example, Bact. denitrificans). Compare Burri and Stutzer (C. B. L. I, 257, 350, 392, 422); Weissenberg (A. H. XXX, 274).

For the demonstration of the denitrifying action of bacteria, there is added to a liter of ordinary bouillon 2.5 gm. of sodium nitrate or, better,—since only thus are *all* denitrifying varieties recognized,—*sodium nitrite.*

As was first completely demonstrated in my institute by eissenberg (Burri and Stutzer had made some similar servations), the reduction of nitrite to nitrogen is much omoted by the exclusion of oxygen, and is markedly or mpletely inhibited by very free entrance of oxygen rowth in shallow layers of fluids or with air passing rough). The organisms thus break up the nitrite to obin oxygen, and there thus originate considerable quanies of NaOH or Na_2CO_3, so that the fluid becomes rongly alkaline.

The test for denitrification is best made with fermentation tubes (p. 90), as the entrance of oxygen is here interfered with; still, usually test-tube cultures suffice. There occurs an abundant production of gas, which is not absorbed by KHO (not CO_2), nor by KHO and pyrogallic acid (not O), and does not burn (not H or hydrocarbons), therefore is nitrogen.

According to Stoklasa, the denitrifying action of bacteria is most pronounced in nutrient materials which, like decayed vegetable matter, straw, and manure, contain abundantly the pentose, xylose ($C_5H_{10}O_5$). Recent literature upon denitrifying varieties: H. Jensen (C. B. L. IV, 401), Künnemann (C. B. L. IV, 906). Here are also described further denitrifying bacteria: Bact. agile Amp. and Gar., Bacillus Schirokikhi Jensen, Bact. filefaciens Jensen, Bact. centropunctatum Jensen, Bact. Hartlebii Jensen, Bact. nitrovarum Jensen, Vibrio denitrificans Sev.[1] Most do not liquefy gelatin and are also able to break up nitrate without the aid of synergetic bacteria. The practical significance of the denitrifying organisms is very great. They rob the soil, manure, etc., of the nitrates and nitrites which are so necessary for the nourishment of plants, and so are powerful enemies of agriculture.

12. Assimilation of Nitrogen.

While, according to our present knowledge, none of the higher families of plants are able to assimilate nitrogen from the air, this property occurs in one variety of bacterium, **Bacillus radicicola** Beyerinck. This bacterium occurs in the small root-tubercles of various leguminous plants[2] (peas, clover, etc.), and may be cultivated from them. It grows poorly or not at all upon the usual nutrient media, but well upon an infusion of pea leaves, to which is added 7% gelatin, 0.25% asparagin, and 0.5% cane-sugar. It does not liquefy gelatin, does not form spores and is non-motile. From the various families of leguminoses various forms of bacteria are obtained, whose morphologic differences, so far as generally definitely proved, appear very slight. Yet every race of bacterium is especially adapted to one family of leguminose, and not every race is able to cause tubercle formation on every family of leguminose. There are, however, "neutral" tubercle-forming bacteria free in the soil, especially adapted to no family of leguminose and able to produce tubercle formation in very different families of leguminoses.[1]

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[1] According to Severin (C. B. L. III, 504), there are yet many more denitrifying varieties; for example, Bacillus subtilis (or closely related varieties), Bacterium indicum, and a coccus.

[2] Regarding the tubercles of the alder which assimilate nitrogen, and their inclusion of fungi, see Hiltner (C. B. L. II, 97)

With the aid of these root-tubercles which are due to the immigration of the root-bacteria, the leguminosæ are capable of thriving upon relatively sterile soil very poor in nitrogen. The bacteria increase in the tubercle and assume bizarre forms, forked, star-shaped, etc., then die out and are absorbed, the plant thus receiving the benefit of the nitrogenous contents of the tubercle. If one ploughs the luxuriantly growing legumines (lupines) into sandy soil (green manuring), the latter is generally so enriched with nitrogen that plants can now thrive that are dependent upon the store of nitrogen in the soil (wheat, etc.). Consult Stutzer regarding the question of formation of tubercles (C. B. L. I, 68; II, 650 and 665).

Mazé (A. P., 1897, No. 1, 44) has undoubtedly shown that nitrogen is also assimilated by pure cultures of bacteria (infusion of beans, 2% cane-sugar, 1% chlorid of sodium, 15% gelatin) accompanied by great consumption of sugar, and also in fluids poor in nitrogen containing 2.6% of sugar. The free passage of air over the culture is very important.

For a long time cultures of the bacteria of legume-tubercles have been sold for the fertilization of soils poor in bacteria and nitrogen, but with sufficient mineral constituents, under the name "**nitragen.**" (Compare Wollny, Vierte Sitzung des bayer. Landwirtschaftsrats, 1898, Heft II.) Recently a preparation known as "**alinit**" has been advocated to increase the yield of wheat. It is a dried potato preparation, which is impregnated with very abundant spores of the so-called Bacillus Ellenbachensis, identical, according to Lauck, with the Bacillus subtilis, or, more properly, according to Stoklasa, with the

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[1] Regarding this point, lately defended especially by Nobbe and his pupils, it must be mentioned that other authors, as, for example, Gonnermann, maintain a *specific* difference for the various tubercle-producing bacteria. As we have made no original investigations in the matter, we desist from further details.

Bac. megatherium. According to the latter, this fertilizer acts as follows: The Bacilli mycoides and megatherium, in the first place, in a nutrient medium poor in nitrogen store up atmospheric nitrogen in the soil; in the second place, they change the nitrate into ammonia and so protect it against the denitrifying organisms. Stoklasa (C. B. L. IV, 817). Lauck saw practically no value in alinit (C. B. L. V, 20); Stoklasa asserts very good results by many observers (C. B. L. V, 350).

Recently the question has been advanced as to whether nitrogen-assimilating organisms are not more widely distributed. Winogradsky has shown in the case of a Cl. Pasteurianum, closely related to the Clostridium butyricum, that it utilizes the energy obtained by the fermentation of sugar in the building up of organic substances from atmospheric nitrogen. Similar observations have been made with molds and a spirillum.

13. Formation of Acids and Alcohol from Carbohydrates.

As pointed out by Theobald Smith (C. B. XVIII, 1), the formation of *free acids* is only possible upon **nutrient media containing sugar.** The acid formation in ordinary bouillon only occurs because of the presence of grape-sugar (very small amount originating in the meat).[1] According to Smith, all obligate or facultative anaerobes form acids from sugar, while the strict aerobic varieties either do not at all or only so slowly that the acid formation is concealed by a corresponding production of alkali. Before knowing of this investigation we had determined that *all* those varieties represented in the atlas which we examined (about sixty) produced more or less free permanent acids in peptone bouillon containing 1% grape-sugar (compare Table I). In connection with the production of acids, perceptible gas-formation was either present or absent. Intense acid production may cause the death of cultures (for example, Bact. coli, Bact. vulgare, etc.). Hellström recently showed that sugar, since it strongly favored the production of acid, especially in poor nutrient media (bouillon without peptone), can shorten enormously the duration of the life of cultures. In peptone-free bouillon, 0.1% of sugar sufficed to kill the cholera vibrio in a few days, 0.2% the Bact. typhi, and 0.3% most bacteria. In solutions rich in peptone the sugar produced less harm.

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[1] According to Th. Smith, 75% of commercial beef contains significant amounts of sugar (up to 0.3%). Regarding the removal of this sugar, consult the Technical Appendix under the preparation of nutrient media.

Since by many varieties the acid-production with the reduction of sugar is very rapid and intense, one designates this metabolism, brought about at the expense of the carbohydrates, as **fermentation** (compare p. 64). Because not rarely gas is produced in abundance, this designation also seems proper to the laity.

If, after the sugar is exhausted, the quantity of acids produced is not such as to kill the bacteria, then in the nutrient medium, now free of sugar, other metabolic processes occur and the acids are neutralized and the reaction becomes even alkaline.

Among the acids produced (besides the carbonic acid, to be spoken of under "gas-formation") the most important, so far as we know, is **lactic acid**; almost always there are at least traces of **formic acid, acetic acid, propionic acid, butyric acid,** and also not rarely some **ethyl alcohol, aldehyd,** or **acetone.** More rarely lactic acid is absent and only the other acids are produced.

For **obtaining and separating the acids** one proceeds somewhat as follows: One-half liter of peptone bouillon containing from 2% to 5% of grape- or milk-sugar is placed in liter flasks, and about 10 gm. of carbonate of calcium added to each. The acids produced unite with the calcium carbonate as soluble salts, and carbonic acid escapes. The reaction of the fluid remains neutral, and that is the principal thing; a strong acid reaction would prematurely hinder further growth of the bacteria.

When growth ceases (after eight to fourteen days), the insoluble carbonate is filtered off, and from the fluid, with neutral reaction, the **alcohol, aldehyd,** and **acetone,** etc., present are removed by distillation, thus reducing very much the amount of fluid. The three mentioned substances are tested for together by Lieben's iodoform reaction. To the slightly warm fluid in a test-tube are added five to six drops of a pure 10% aqueous solution of caustic potash; then drop by drop a weak solution of iodid of potassium is added until a brown color appears, and the latter is again dissipated by a drop of potash. The presence of iodoform is proved by the characteristic odor and, microscopically, by the small six-sided iodoform plates. For the differentiation of alcohol, aldehyd, and acetone, consult Vortmann, Analyse organ. Stoffe, 1891.

Then one acidifies strongly with phosphoric acid, and with the aid of a current of steam distils off the **volatile acids.** The distillation must be long continued, as the complete separation of the volatile acids is difficult. The non-volatile lactic acid (together with some succinic acid) remains behind and is separated by repeated shaking with pure ether, the ether then being distilled off.

The **lactic acid** obtained is always ethylidenlactic acid, $CH_3.CHOH.COOH$, which occurs in two stereoisomeric forms: (1) **dextrorotatory** with levorotatory zinc salts; (2) **levorotatory** with dextrorotatory zinc salts. If, as is frequently the case, almost equal molecules of levorotatory and dextrorotatory lactic acid are present, then the mixture is optically inactive and is the so-called "fermentation lactic acid." I believe that often both lactic acids originate from sugar, but that many bacteria use up one acid exclusively or principally, while others appropriate the other acid. Thus may occur now a uniform mixture of both acids, now one acid exclusively or preponderantly.

Since Schardinger (Mitt. f. Chem. XI, 545) first discovered the previously unknown levorotatory lactic acid as a product of a short bacillus from water, many investigations have been made, especially by the pupils of Nencki and Rubner, regarding the lactic acids formed by different varieties of bacteria, with the hope of utilizing the results in differential diagnosis.

For the methods for determining which lactic acid is present, consult Nencki (C. B. IX, 305) and Gosio (A. H. XXI, 114). They have to do with the determination of polarization and the water-content of the zinc salt.

The most important results of the investigations are:

	INACTIVE LACTIC ACID.	DEXTROROTATORY LACTIC ACID (PARALACTIC).	LEVOROTATORY LACTIC ACID.
Bac. coli[1]		+	
Bac. Bischleri	+		
Bac. typhi			+
Microc. acidi paralactici		+	
Vibrio choleræ (Calcutta)			+
Vibrio choleræ (Massina)			
Vibrio Metschnikovi			
Vibrio danubicus			
Vibrio "Wernicke", I, II, I-I			
Vibrio "Dunbar"			
Vibrio proteus			
Vibrio Weibel			
Vibrio Bonhoff *b*.	+		
Vibrio berolinensis			
Vibrio aquatilis			
Vibrio tyrogenes		+	
Vibrio Bonhoff *a*			

While at present these results are not of much value, yet a continuance of these theoretically interesting studies is desirable. (Compare special part, under Vibrio choleræ and Bact. coli.)

Various bacteria—often, however, insufficiently studied morphologically or biologically—are able to produce **butyric acid, butyl alcohol,** or both from carbohydrates.

For a review of these varieties see Baier (C. B. L. I, 17). Compare in special part: Bac. butyricus Hüppe, Bac. butyricus Botkin, Clostridium butyricum, etc.

In connection with the fermentation of sugar, **decomposition of cellulose** may be mentioned as caused by various bacteria. It occurs especially in the gastric and intestinal contents of herbivora, and also in quagmire, and forms marsh-gas as its striking product.

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[1] The statements regarding the coli group are from Nencki (C. B. IX, 305); regarding the typhoid, from Blachstein; regarding the cholera group, from Kuprianow (A. H. XIX, 283, 291) and Gosio (A. H. XXI, 114).

Unfortunately the fermentation of cellulose by bacteria is insufficiently studied. So much seems certain, that at least one anaerobic variety converts **cellulose** into **marsh-gas and carbonic acid.** Yet Van Senus maintains that the anaerobic "Bacillus amylobacter," isolated by him, attacks cellulose only in symbiosis with another small bacillus. (Compare the résumé by Herfeld, C. B. L. I, 74, 114, and also the special part.)

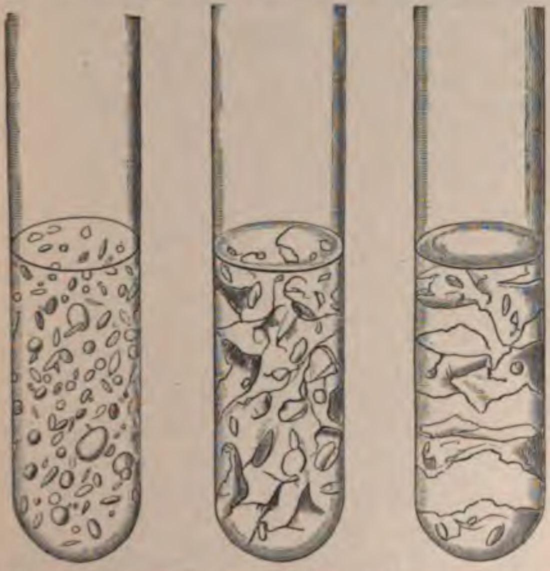

Fig. 11.—Bacterium coli upon sugar-agar, after twelve, twenty-four, and forty-eight hours.

14. Gas-production from Carbohydrates and Other Fermentable Bodies of the Fatty Series.

The **only** gas eventually arising in visible quantity[1] in nutrient media which **contain no sugar** is **nitrogen** (compare page 82).

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[1] Sulphuretted hydrogen and ammonia can scarcely occur in visible quantity.

If **sugar** is broken up energetically by bacteria, gas-formation may be absent, only lactic or acetic acid being produced (for example, Bac. typhi on grape-sugar), but very often an enormous production of gas occurs, especially if air is excluded. About one-third of the vigorous acid-forming varieties produce abundant gas, which consists always of **carbonic acid,** with a constant admixture, according to Smith (C. B. XVIII, 1), of **hydrogen. Marsh-gas** appears to be rarely formed (aside from the bacteria causing fermentation of cellulose). Compare in special part: Bact. brassicæ acidæ.

To determine **whether gas is formed,** the shake-culture in 1% grape-sugar agar is very useful (Fig. 11). After twenty-four hours (if incubator temperature is available, often after six to twelve hours) the agar is beset by gas bubbles or cleft by numerous deep holes and cracks. If it is desirable to collect and measure the gas, to investigate he curve of the intensity of gas production, or to analyze , the gas is best collected after the method of Th. Smith in fermentation tube, such as has been long employed in hysiologic and pathologic chemistry (Fig. 12).

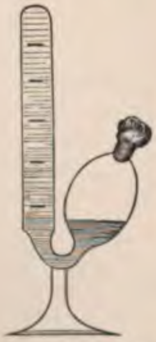


Fig. 12.—Fermentation tube.

The tubes, which preferably have the same form, are filled with 1% grape-sugar peptone-bouillon (without air-bles) and sterilized in the steam sterilizer.

r inoculating with a platinum loop, they are kept in the incubator and the following observations made (Th. Smith):

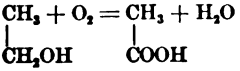
1. If cloudiness occurs only in the open limb, then one is dealing with an aerobic variety; if only the fluid in the closed limb becomes cloudy, while that in the bulb remains clear, then an anaerobic variety is present.

2. One notes the daily gas-formation by an ink mark, and, if the tubes are graduated, in four to six days, after the gas production has ceased, the percentage of gas formed on each day can be determined.

3. A rough **analysis of the gas** is made. With this object in view, after indicating the quantity of gas produced by means of a mark, the open bulb is completely filled with 10% solution of caustic soda and closed tightly with the thumb. The fluid is shaken thoroughly with the gas and allowed to flow back and forth from bulb to closed branch and the reverse several times. Finally, the gas is allowed to again rise in the closed limb, and after removing the thumb, the new volume of gas is read. The part removed consists of CO_2; that remaining consists of nitrogen, hydrogen, and marsh-gas. For quantitative analysis of these gases it is best to employ Hempel's gas pipet. (Compare Cl. Winkler, Lehrbuch der techn. Gasanalyse, Freiberg, 1892.) The principle of the method is that hydrogen mixed with oxygen carried over red-hot palladium asbestos is converted into water, thus disappearing; marsh-gas in a red-hot platinum capillary is burned up to carbonic acid, and as such is determined; what is left is nitrogen. With some practice the investigation is easy and accurate.

15. Production of Acids from Alcohols and from Other Organic Acids.

The transformation, in weak solutions, of ethyl alcohol, under energetic consumption of oxygen, into acetic acid by the Bact. aceti or its nearest relatives has long been known (compare p. 66 and special part):



$$\begin{matrix} CH_3 & + O_2 = & CH_3 & + H_2O \\ | & & | & \\ CH_2OH & & COOH & \end{matrix}$$

Also higher alcohols: Glycerin, dulcite, and mannite are changed into acids; glycerin as constantly as sugar is (v. Sommaruga, Z. H. xv, 291).

Finally, numerous results have been obtained regarding the transformation of acids of the fatty series (or their salts) into other fatty acids by bacteria. Unfortunately, the earlier observations were made without the employment of such pure cultures as satisfy the present demands. Usually **lactate, malate, tartrate, citrate,** and **glycerate of calcium** were employed, while almost always mixtures of acids were obtained as a result of the activity of the bacteria. Among these, **butyric, propionic, valerianic,** and **acetic acid** play the principal rôle; often also there occur **succinic acid, ethyl alcohol,** and more rarely **formic acid.** Of gases, **carbonic acid** and **hydrogen** occur especially.

Such investigations were formerly carried out particularly by Fitz, and more recently they have been performed on an extensive scale with undoubtedly pure cultures and with interesting results by P. Frankland.

Here only two illustrations are given: Pasteur found that anaerobic bacteria converted lactate of calcium into the butyrate:

$$\underbrace{2(CH_3 - CHOH - COO)_2Ca}_{\text{Lactate of calcium.}} =$$

$$CO_3Ca + 3CO_2 + 4H_2 + \underbrace{(CH_3 - CH_2 - CH_2 - COO)Ca}_{\text{Butyrate of calcium.}}$$

According to P. Frankland, the Bacillus æthaceticus Fitz forms from the glycerate of calcium, $(CH_2OH\text{-}CHOH\text{-}COO)_2Ca$, ethyl alcohol, acetic acid, carbonic acid, and hydrogen.

5. THE PATHOGENIC ACTION OF BACTERIA (PATHOGENESIS, PREDISPOSITION, RESISTANCE, IMMUNITY).

I. How Do Bacteria Act Pathogenically?

If micro-organisms enter the tissues or blood of an animal, there occurs an infection if, at the same time—

1. The micro-organisms can remain alive and increase within the host.
2. If the micro-organisms produce substances which are injurious to the host.

Theoretically, the blood and organs of healthy animals contain no germs, yet we must accept the fact that often solitary streptococci, tubercle bacilli, etc., are present in the healthy body, circulating in the lymph- and blood-stream and becoming fixed at loci minoris resistentiæ, from which places they extend further. Perez found, in a systematic examination of healthy bodies, that only the lymph-glands contain bacteria, but here the bacterial flora was very rich.[1] In dead animals after sixteen to twenty hours at room temperature or after five to six hours in the incubator, bacteria are found in the blood and organs (Trombetta), having for the greater part wandered from the intestine. In the most frequent artificial method of infection, *i. e.*, subcutaneous injection, the bacteria are absorbed through the lymph-stream, and in part are held back in the lymph-glands, weakened in their virulence and even killed; but if the organisms are strongly "pathogenic," they resist total destruction, and, on the contrary, begin to increase a few hours after entrance. Regarding the method of destruction of bacteria, compare page 97.

Wherever we inspect the character of the pathogenic action of bacteria, they are found to operate through chemical substances which they produce or which are produced from them in the animal body. Thus far we are able to understand the action of only those bacteria which in cultures form poisonous substances, by means of which we can also reproduce the characteristic picture of the disease more or less accurately. Bacteria of this class are: Bac. tetani and Corynebact. diphtheriæ, Streptococcus pyogenes, Micrococc. pyogenes, Vibrio choleræ, etc. There has already (p. 73) been given a sketch of what we know chemically about these poisonous substances.

On the contrary, the means for an explanation upon a chemical basis are almost entirely absent as yet in a series of important infectious diseases, among which are, for example, anthrax (Conradi, Z. H. XXXI, 287) and swine erysipelas. The filtrates through porcelain from the most virulent cultures are without effect; cultures carefully devitalized by short heating or brief action of chloroform, when injected produce only the general effects of protein (fever), and still in these diseases poisoning by the metabolic products of bacteria is certainly present.

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[1] It may be mentioned here that often a considerable number of micro-organisms are also rapidly secreted through the bile and urine without any perceptible injury to the organ being demonstrable (Biedl and Kraus, Z. H. XXVI, 353), yet many authors assume, in these cases, at least microscopic rupture of vessels (Opitz, Z. H. XXIX, 505).

It is to be noted as an interesting finding that Petri and Maassen (A. G. A. VIII, 318) could demonstrate sulphuret-hemoglobin lines in the fresh blood and edematous fluid of pigs sick with swine erysipelas,—an indication that at least sulphuretted hydrogen poisoning is *concerned* in the death of the animal. Also in malignant edema a similar demonstration is successful. Hoffa has tried to conceive of rabbit septicemia as poisoning by methylguanidin (Langenbeck's Archiv, 1889, p. 273). Emmerich and Tsuboi (Münch. med. Wochenschr., 1893, No. 25, 473)—but certainly without receiving any support—attempt to explain cholera as poisoning by nitrite.

Certainly these explanations possess great interest, but often they do not suffice, for at least there occur, besides the just mentioned poisoning processes, also specific changes in the blood and tissues of animals, which are proved, among other things, by the generation of **specific protective substances ("anti-bodies").**

II. Variation in the Virulence of Bacteria.

The **virulence** of bacteria is just as **variable** as are all the other functions (chromogenesis, fermentation, etc.). It is best preserved by continual inoculation of the bacteria from one susceptible animal to another. It may also be accomplished in many varieties by rather frequent transfer (about monthly) from one artificial nutrient medium to another, but it is best to pass the bacteria through an animal from time to time. On the contrary, the virulence usually suffers if, with infrequent transfer, the culture remains long in contact with its accumulated metabolic products.

Reduction of virulence is not difficult to bring about:

(*a*) By growing at a somewhat too high temperature anthrax becomes completely avirulent—at 42.5° in three to four weeks, at 47° after several hours, and at 50° to 53° in a few minutes. By a proper regulation of the reduction through the action of heat the anthrax bacillus can be attenuated to such a degree that it will kill only mice, or mice and guinea-pigs, or, besides these, also rabbits. Also *spores* (symptomatic anthrax) may be attenuated by means of dry heat or short careful steam disinfection (Kitt).

(*b*) By growth upon unfavorable nutrient media. An addition of phenol ($\frac{1}{500}$) and of bichromate of potassium (0.04% to 0.02%) to nutrient media is employed to attenuate the anthrax bacillus, and iodin trichlorid the diphtheria bacillus. Growth on media containing sugar always, in time, lowers the virulence (Levy).

(*c*) By the action of sunlight, compressed oxygen, etc.

(*d*) By repeated passage through unsuitable animals. Swine erysipelas becomes much less virulent after repeated passage through rabbits, the variola organism (although not a bacterium) after passing through the cow.

It is much more **difficult** to restore a **heightened virulence to attenuated bacteria.** In general it is true that the more rapidly the attenuation is accomplished, the more rapidly will the virulence be spontaneously restored.

Varieties which have gradually (spontaneously, *i. e.*, from the action of their metabolic products) suffered loss in virulence may have their virulence again increased in most cases, but not always, in one of the following ways:

1. Growing in bouillon to which has been added ascitic fluid (streptococci, diphtheria bacilli). (Compare von Dungeren, C. B. XIX, 137, and special part.)

2. By first inoculating especially susceptible animals,—*i. e.*, very young animals of the susceptible variety,—and if these succumb, transferring the causative agent directly with the blood of this animal to an older, more resisting example of the susceptible species, and later to still more resisting species of animals. Each passage through the animal strengthens the virulence, until a certain maximum is reached. Compare also Knorr's experience with the Streptococcus pyogenes.

3. By first infecting susceptible animals with large quantities of fresh bouillon cultures of the variety concerned, the simultaneously introduced metabolic products cooperate to heighten the disposition of the animal for the injected organisms.

4. Together with the bacteria (especially staphylococci and streptococci) there is injected a large quantity of the metabolic products of *Bact. vulgare.* The action is explained as under 3.

5. By the injection, together with the weakened bacterium, also of another which in itself is entirely harmless; for example, with the Bacillus œdematis maligni or Bacillus anthracis, the Bact. prodigiosum.

6. The injection of the culture mixed with an injurious substance not of bacterial origin; for example, lactic acid. With the Bac. œdematis maligni increased pathogenic properties have thus been ob-

served, due to a local lowering of the bacteria-resisting property of the inoculated animal at the point of the injection.

III. Predisposition and Congenital Immunity (Resistance).

The **susceptibility** of various species and single individuals to various infectious diseases **from birth** is striking and not easily explained.

In the first place, certain species of animals are naturally **absolutely immune**[1] to certain infectious agents; for example, man to murrain, cattle to glanders, all experimental animals to syphilis, malaria, and gonorrhea.

Other diseases occur at least only rarely and with difficulty in certain varieties of animals; for example, anthrax in certain races of pigeons, rats, and sheep. Here there exists a **relative** immunity. Relative immunity is usually proportionate to the strength and maturity of the animal. All kinds of injury (hunger, cold, overexertion, incorporation of certain poisons) lessen the immunity,—considerably increase the disposition,—so that a large number of animals, weakened in this way, succumb to a subsequent infection.

It is, therefore, necessary, with **every newly isolated** variety of bacterium of which one desires to ascertain the **pathogenic action,** to include in the test the **most varied animals,** if the first chosen animal gives a negative result. Our principal experimental animals are: White house mice, white rats, guinea-pigs, rabbits, chickens, pigeons, and, for special purposes, monkeys. More rarely we employ gray house mice, rats, field mice, gophers, dogs, cats, cows, sheep, pigs, and horses. The most desirable experimental animal is the guinea-pig, but it requires good care. It is recommended by its convenient size, docility, and limited consumption of food. Animal diseases are usually much easier to investigate and explain absolutely than human diseases, because the susceptible experimental animal is always at command. In difficult cases experiments in infection have also many times been carried out on man.

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[1] It is especially noteworthy that very closely related varieties often conduct themselves very differently in this regard; as, for example, the glanders bacillus easily infects field mice, but not house mice; anthrax bacillus kills house mice with almost absolute certainty, but is not pathogenic for the rat, etc.

The **causes of congenital immunity** (**resistance,** Buchner) lie in protective arrangements of the organism, regarding which I cannot here speak exhaustively. Only so much is given as is fairly in accord with all the facts, corresponding to the views formulated by Buchner as a compromise to the various opposed opinions. Upon invasion of a resisting organism by pathogenic germs, a part is destroyed by pre-existing protective substances (**alexins**) which are in solution in the serum (originating from leukocytes); another part is destroyed by alexins, which are produced from leukocytes (also from other tissues eventually) under the influence of the bacteria.[1]

In the body the leukocytes appear to be able to live while supplying these secretions; in the test-tube certainly it is only possible to obtain bactericidal substances from leukocytes with certainty through injury (freezing, distilled water, foreign serum, toxins) (Schattenfroh, A. H. xxxv, 135). The serum acts more strongly upon pathogenic than upon non-pathogenic varieties (Leclef); also spores may be destroyed.

Part of the organisms destroyed by the alexins are supplementarily taken up by leukocytes, but, moreover, it is undoubtedly true that at least some organisms are, while living, devoured by leukocytes. Metschnikoff and his pupils, moreover, insist (without, in recent times, contesting the significance of the alexins) that the latter phenomenon (**phagocytosis**) is of the highest import in immunity. Denys (C. B. xxiv, 685) has directly shown that pathogenic streptococci are scarcely at all, while, on the contrary, non-pathogenic ones are very rapidly devoured by leukocytes; the absence of phagocytosis here only concerns the pathogenic streptococci.

So far the alexins have not been isolated; they are very unstable, and are rendered inactive by a temperature of 55° or by the action of sunlight.

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[1] Recently, however, Sawtchenko and Schattenfroh have communicated investigations, from which it follows that, at least in some cases, the poly- and mono-nuclear leukocytes contain no alexin, in spite of strong bactericidal action of the serum (Schattenfroh, Münch. med. Wochenschr., 1898, No. 12, 353).

Recently statements regarding **heat-resisting substances** which are injurious to bacteria, and which differ from alexins, have multiplied (Löwit, Bail). These appear to belong to the nuclein compounds,[1] and are particularly produced from lymph-glands.

Besides the bactericidal alexins, frequently agglutinin and antitoxic substances (for example, against diphtheria) are demonstrable in healthy individuals as congenital.

An **increase** of the **congenital resistance** to various infectious diseases has been sought and obtained in many ways. Favorable effects sometimes against one, sometimes against several infectious diseases have been obtained by a number of investigators by injecting animals with thymus extract, spermin, abrin (poisonous albuminous bodies from the paternoster pea), papain (albumin-dissolving ferment from papaw-tree); also cinnamic acid, trichlorid of iodin, carbonate of sodium,[2] etc.

Recently there has been discovered a protective action against tetanus and botulism through injections of brain substance (Wassermann and Takaki, Kempner), and against typhoid through injection of spleen substance (Aujeszky).

IV. Acquired Specific Immunity and Its Causes.

There is a **sharp contrast** to this heightened resistance, according to most authors, and the **specific immunity against a definite disease,** which originates when an attack of the disease is acquired and recovered from spontaneously or when it follows purposeful injection (**active immunity**):

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[1] H. Kossel has shown that less than a 0.5% solution of albumin precipitating nucleinic acid (obtained from calves' leukocytes) has strong bactericidal action. Yet the nucleinic acid could scarcely explain the alexin action. Previously (1893) Vaughan and MacClintock recognized nuclein as destructive to germs.

[2] According to Fodor, an increase in the alkalinity of the blood is accompanied by an increased resistance to many bacteria, but, according to Fodor and Rigler, also every introduction of toxin is followed by a decrease, and every introduction of antitoxin by an increase, in alkalinity (C. B. XXI, 134).

1. With naturally or artificially attenuated infectious agents of similar kind; or

2. With devitalized cultures of the micro-organisms concerned.

So far we know of two entirely different causes for this immunity.

A.—Poison Resistance (Specific Poison Immunity).

After recovery from a series of infectious diseases (diphtheria, tetanus, botulism) which have this in common, that during their course very active poisons are produced by the bacteria, there are found in the blood, and especially in the serum, characteristic substances which are actively antagonistic to the poisons (**antitoxins**). The antitoxic serum protects as well if it is used before the introduction of the poisonous culture (**it immunizes passively**) as if it is injected at the time of, or subsequent to, the infection (**it heals**). It operates as well against the introduction of the toxins of the concerned bacteria as (in larger doses) against the introduction of living cultures.

As yet little is known regarding antitoxins, but they are more resistant than the alexins to injurious influences. Thus the tetanus antitoxin bears very well without being destroyed: a temperature of 60°, also 70° to 80° for a shorter time, the action of sunlight (yellow better than blue rays), and putrefaction. Brieger and Ehrlich have obtained diphtheria antitoxin from the milk of goats, immune to diphtheria, in solid form,—whether it is an albuminous body or adheres to albuminous bodies, is still unknown. The antitoxins are best separated by means of zinc chlorid, but so far can not be freed from the final traces of zinc (Brieger and Boer, Z. H. xxi, 266).

Regarding the action of antitoxins and toxins upon each other the following is now known. As Behring and Kitasato supposed, a toxin solution in a test-tube with a sufficient quantity of antitoxin added is completely inactive, because toxin and antitoxin mutually neutralize each other (somewhat as base and acid). Buchner explained this neutralization as only apparent, and maintained that both antitoxin and toxin continue together, but mutually hide each other because they influence the same animal organism in opposite ways, somewhat like atropin and muscarin. Now Buchner (Münch. med. Wochenschr., 1899, 523), especially since the work of Knorr (Z. H. XIII, 407), has accepted essentially the views of Behring. The principal proofs for these views are: (1) Dilute solutions of toxin and antitoxin neutralize each other more slowly than concentrated ones (in higher dilutions many chemical reactions occur slowly). (2) Equalized toxin and antitoxin mixtures after heating or long storage remain non-toxic, while toxin is much more resistant than antitoxin. (3) Animals into which are injected subcutaneously corresponding mixtures of toxin and antitoxin, after two hours show no antitoxin in the blood, although the toxin is much more rapidly absorbed. Finally, it is very noticeable that **ricin** (poisonous albuminous body from ricinus seeds) and **antiricin** act also **antagonistically** upon dead bodies **in vitro** (diluted blood) as if they neutralized each other; the antiricin increases the coagulating action of ricin (Ehrlich, Fort. der Med., 1897, 41). Similarly rattlesnake poison (cobra toxin) in vitro dissolves red corpuscles, while cobra antitoxin hinders this action. Stephens-Meyers (London Lancet, Mar. 5, 1898, 644).

As to opposing statements: Take an animal that is protected against a mixture of toxin and antitoxin, but not also protected against two, four, six, or ten times the quantity of the mixture (Bomstein and others). Cobbett and Kanthack assert as their positive opinion, at least in the case of diphtheria, that, if the toxin has been completely neutralized, even ten times the quantity of the mixture could be tolerated (C. B. XXIV, 129), but, naturally, if the mixture contains a slight excess of toxin, ten times the quantity would be injurious.

Reference must be made to the incompletely understood fact that to a neutralizing mixture of toxin and antitoxin a very large quantity of toxin must be added before slight toxic action results from its injection, but that also very large quantities of antitoxin are required to again overcome this weak toxic action.

thal, the "**toxin-binding group**" of the toxin-susceptible albumin molecule.

The recent experiences of Wassermann, Behring, Blumenthal, Metschnikoff and Maria, and especially of Knorr (Münch. med. Wochenschr., 1898, Nos. 11 and 12), accord very well with this explanation. Thus, what occurs in the case of tetanus may be presented somewhat as follows:

If one introduces tetanus poison into an animal susceptible to tetanus (guinea-pig), after a time the poison disappears from the blood and becomes insolubly fixed by the chemical constituents of the ganglia of the spinal cord, and is therefore no more obtainable from the spinal cord. The binding of the poison by the spinal cord leaves it diseased; the poison-binding cells become functionally incapacitated. Wassermann could directly demonstrate that the spinal cord (and brain) can bind tetanus poison, since he showed that mixtures of tetanus toxin and emulsion of spinal cord are non-toxic. It is further interesting that the cord of animals susceptible to tetanus alone possesses this property, the cord of hens, which are immune to tetanus, not at all. The side chains are absent here, and the hen is insusceptible to tetanus for the same reason that its cord is without effect upon the toxins. On the contrary, the cords of animals dying from tetanus contain sufficient antitoxin to protect other animals from the disease. This is no objection, if the first animal died when a certain part of its spinal cells were poisoned through the union with toxin and long before all the poison-binding affinities of its spinal cord were satisfied.

Knorr has shown the identity of the antitoxin and the poison-fixing substance of the spinal cord by demonstrating that both possess the same susceptibility to injurious influences.

Where, by careful repeated poisoning of an animal until the surplus supply of antitoxin is dissolved in the serum, the phenomena may be represented, according to Ehrlich, somewhat as follows: If, with the first cautious introduction of a small amount of tetanus poison only a small part of the toxophoric side chains is fixed, then only a few cells are injured, or many cells are so slightly injured that no disease symptoms occur. The side chains united with toxin are thrown off and replaced by the cells. This repair is always accomplished more rapidly, the more frequently and extensively the side chains are torn off by the toxins. Finally, the cells produce more side chains than can remain connected with them, and they are discharged into the blood as antitoxin. Knorr finds this explanation of the facts unsatisfactory, and claims that, while in one part of the diseased organism the antitoxin groups are fixed by toxin, the unpoisoned cells containing antitoxin are stimulated to a larger production of this substance.

From this essential independence of antitoxin from the chemical composition of the toxin one can understand that a specificity of antitoxin does not strictly exist. For example, tetanus and rabies antitoxin also protect against cobra poison (Calmette, A. P. IX, p. 225), and, according to Tizzoni, sterile non-toxic cultures of pneumococcus in rabbits' blood protect against tetanus poison (C. B. XXIV, 904).

The **value of antitoxic sera** is expressed as follows:

1. *After Behring.* He designates as a **normal poison** a toxin solution of which 0.01 c.c. is sufficient to kill a guinea-pig weighing 250 gm. within four days. The toxin is always injected in 4 c.c. of water just beneath the skin. Of this normal diphtheria toxin (DTN) 1 c.c. is sufficient to kill one hundred guinea-pigs, each weighing 250 gm., or 25,000 gm. in weight of guinea-pigs. Behring expresses this as follows:

The DTN has a working value of 25,000; it kills 25,000 times its weight of guinea-pigs. A toxin ten times as strong is represented as DTN^{10}; a ten times weaker one, as $\frac{DTN}{10}$.

The quantity of antitoxin that is required to just pro- ect 25,000 gm. weight of guinea-pigs from the minimal al dose of poison is called one immunizing unit.

If an immune serum contains in 1 c.c. one immunizing unit (IE),—*i. e.*, neutralizes 1 c.c. DTN,—then it represents a **normal antitoxin** (DAN).

To determine the strength of an immune serum, 1 c.c. of normal toxin is mixed with increasing quantities of the serum; the quantity of the serum which suffices to neutralize it—for example, 0.1 c.c.—contains one immunizing unit, or the serum contains 10 IE to the c.c., and is then ten times DAN, and is represented thus, DAN^{10}.

To cure a sick man, usually 600 to 1800 IE are employed, which are contained in 2, 4, or 6 c.c.; then it is a DAN^{300} that is used.

Recently a dried DA has been produced, of which 1 gm. contains as much as 5000 IE; of this about 0.125 gm. suffices for a single healing dose.

2. *After Ehrlich.* Ehrlich has recently introduced in the institute for testing serum, as a standard for determining values, a very durable dry antitoxin, which contains 1700 IE in 1 gm. A test-toxin is prepared corresponding to this antitoxin, and with this toxin the strength of the unknown serum is titrated. For the crude estimation of the working value of a serum, a toxin can be prepared corresponding to a higher serum of guaranteed strength of IE, and the unknown serum be titrated with this toxin. Because of the numerous cautions to be observed, serviceable results can be obtained only by experts. Compare Ehrlich, Klin. Jahrbuch, Bd VI.

B. Bacteria Resistance (Specific Bacterial Immunity).

While the antitoxins antagonize the toxins of the bacteria in an active manner, they are able, according to older observations not at all, according to more recent observations[1] only to a slight extent, to kill bacteria—*i. e.*, to act as bactericides. On the **contrary,** in a **second group of infectious diseases** (typhoid, cholera, swine erysipelas) **the immunity depends upon the bactericidal action of the**

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[1] According to van de Velde (C. B. XXII, 527), strong antidiphtheria serum also possesses considerable bactericidal action; similar double action is presented also by various other immune sera; for example, antipyocyaneus serum. Compare also Emmerich and Löw, p. 110.

sera. If an animal has recovered from an infection with one of these diseases, or has received in a proper manner the devitalized cause of the disease, then its body, especially its blood, contains materials which even in high dilutions possess intense and even specific destructive action upon the micro-organisms concerned. Also in man suffering from the infectious diseases such bactericidal bodies develop in the blood; indeed, they are often present from the eighth to the fourteenth day of the disease, and may persist after recovery for weeks, months, or even years. An active immunity of this kind has been successfully produced in man against cholera and pest. In these diseases a positive immunity is conferred by the injection of devitalized culture masses (Ferran and Hafkine).

The bactericidal serum from animals actively immunized to a high degree has been employed with good results to produce passive immunity in other organisms in various diseases (for example, swine erysipelas by Emmerich). Usually there is injected, besides the serum, also the normal or attenuated specific agent.[1]

To obtain serum one proceeds as follows:

1. *Collection of human serum.* The finger is carefully cleansed with soap and water, alcohol and ether, and a small puncture is made with a strong needle on the front and to the side at the end of the finger, so that three or four large drops of blood are obtained. The blood is collected in a U-shaped capillary tube, and the ends closed with wax or sealing-wax. As soon as possible the sample is centrifugated in a high-speed hand centrifuge, the wax being previously removed and the open ends of the tube being turned toward the center. The centrifugation is continued for ten minutes. After it is completed each limb of the tube contains about 1 to 1.5 cm. of clear serum. A piece 1 cm. long is now measured off in each branch and, after marking it lightly with a file scratch, the tube is

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[1] In mouth and foot disease, for example, Löffler, for the purpose of producing immunity, injected a mixture of serum from the recovered animal together with the virulent contents of the vesicle of the sick animal. Kolle, in immunizing against murrain, employs the simultaneous injection (in two different places) of serum from a recovered cow, and virulent blood from the diseased animal.

broken off; thus two pieces 1 cm. long and filled with clear serum are obtained.

2. *Collection of serum from animals.* A rabbit is injected with cultures of typhoid or cholera, devitalized by heat, following the detailed directions given in connection with typhoid and cholera. After about ten days the animal is bled from the carotid and the serum separated by standing in a tall cylinder in a cool place or by centrifugation. The measurement of the serum and the diluting fluid is carried out by means of uniformly wide capillary tubes, which are marked off with ink-lines in lengths of a centimeter each. The relative and not the absolute quantities of the materials are thus known. In the following, when I speak of 1 cm. of serum, I mean that contained in a piece of a capillary tube 1 cm. long.

The bacteria are always employed as a suspension in 0.5 c.c. of bouillon, one loopful[1] (2 mg.) of a twenty-four hours' agar culture (37°) being used in its preparation.

The serum is also diluted with bouillon.

For demonstrating the injurious action upon bacteria, we have two methods: The demonstration of the specific agglutinating material according to Gruber and Durham, and the specific bactericidal material according to R. Pfeiffer.

1. *Demonstration of agglutinin,*[2] *after Gruber and Durham* (Münch. med. Wochenschr., 1896, 206 and 285). If it is desired to make a *macroscopic* demonstration (little used), 0.5 c.c. of the suspension of the bacteria is added to 0.5 c.c. of serum diluted fifty times, and it is noted in a very narrow test-tube whether there occurs a visible clumping of the bacteria with clearing up of the fluid. If after ten to fifteen minutes, or at most one hour, no reaction occurs in the incubator, the serum in a dilution of 1:50 is not active upon the variety of bacteria tested, and we may repeat the test with stronger concentrations. On the contrary, the ex-

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[1] The loopful is estimated by weighing the empty and full loop on the needle.

[2] If one employs more bacteria, the action of the serum is weaker. In order to obtain cultures that are readily broken up, the culture is prepared upon agar that has been somewhat dried by being previously kept in the incubator for twenty-four hours.

periment is repeated with more dilute serum if it results positively in the first instance.

More accurate than the macroscopic is the microscopic method, also suggested by Gruber and Durham. In the examination of human blood rarely is there more serum at hand than is required for the microscopic examination. After the serum has been obtained by centrifugation in two glass capillaries 1 cm. in length (see above), the serum from one tube is blown out into a cell by means of a fine tube placed above it, and then has added to it bouillon from 5 segments, each 10 cm. long, of a tube of similar size to that which contained the serum. In a hanging drop, by means of the immersion lens, it is observed whether agglutination of introduced bacteria occurs. This follows, if the reaction is strong, in a few seconds; if the action is weaker, in from ten minutes to one hour. It is observed that the organisms suffer a loss of motion, become somewhat swollen (rarely seen) and cemented together in irregular bunches and clumps. Single bacilli often remain longer motile. If the reaction is not promptly positive, the preparation is kept in the incubator and examined after half an hour and one hour. Positive results after two hours are not of much value. Control preparations without serum, but with bouillon only, should always be prepared by the beginner, so as not to mistake a sedimentation, etc., for agglutination.[1]

If the action occurs with a dilution of $\frac{1}{50}$, then it is a positive reaction, and it can then be determined whether $\frac{1}{100}$, $\frac{1}{200}$, $\frac{1}{500}$, $\frac{1}{1000}$, and $\frac{1}{5000}$ are also active, the necessary dilutions being prepared preferably by further dilutions of the first sample. If no result occurs with the dilution of $\frac{1}{50}$, then the reserved centimeter tube of serum is diluted $\frac{1}{25}$; and if still no reaction is obtained, the diagnosis is absolutely negative. In general it is customary to attach no value to reactions with higher concentrations than $\frac{1}{40}$ to $\frac{1}{50}$. (Compare further under Bact. typhi and Vibrio choleræ.)

The reaction is in a great degree specific (see below).

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[1] Cultures killed with chloroform vapor are likewise agglutinated; also some non-motile varieties, as Streptococcus lanceolatus, Bacterium pestis, and Bact. pneumoniæ, have been caused to agglutinate by specific sera.

Virulent and avirulent cultures are alike affected; even the expressed bacterial cell-juice and, moreover, the germ-free filtered bouillon cultures are precipitated by specific immune sera (Kraus, Wien. med. Pr., 1897, 608).

The paralyzed and clumped organisms are not dead, or only partially so, for after twenty-four hours an active increase of the organisms is often observed. Although the clumps do not dissolve, and at most loosen up, the preparation swarms with actively motile forms. If one adds new bacteria to a preparation in a state of agglutination, they are not affected, the agglutinin having been consumed, and with the addition of new serum agglutination again occurs.

2.[1] *Demonstration of specific bactericidal*[2] *bodies in immune sera according to R. Pfeiffer.* We will employ cholera as an example. If one mixes a suspension of one loopful of virulent cholera culture in 1 c.c. of bouillon with 0.01 c.c. to 0.03 c.c.[3] of cholera-immune serum and injects the mixture into the peritoneal cavity of a healthy guinea-pig, he will observe there, besides paralysis and swelling, death, granular degeneration, and, finally, solution of the introduced germs. In this case a virulent culture must be selected, since avirulent organisms, even without the addition of immune serum, die and are dissolved in the peritoneal cavity. This reaction is specific to a high degree (compare below). To make the examinations, peritoneal lymph is obtained with a capillary pipet through a small opening in the abdominal wall, and examined microscopically every ten minutes for about half an hour to one hour to determine the fate of the bacteria. After this time, if the reaction is positive, nothing more is to be seen of the vibriones except single granules, and these are not always easily found, and the peritoneal contents have become viscid, mucoid, and tenacious. If the result is negative, the peritoneal exudate after an hour contains large numbers of actively motile vibriones. It is recommended that a control test be made upon a second animal with the same bacteria and normal serum. Normal serum at most causes in quantities of 0.1 c.c. and upward a very slight positive reaction—*i. e.*, it causes a few vibriones to undergo granular degeneration (compare R. Pfeiffer and Kolle, C. B. xx, 129).

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[1] A third "specific" serum reaction has been recommended by v. Dungern (C. B. XXIV, 710). A little of the serum from animals which have passed through cholera and staphylococcus infection, and anthrax, exerts a marked inhibitory action upon the liquefaction of gelatin by a portion (1 c.c.) of a liquefied gelatin culture of the same variety. Normal serum restrains it less; the interference with the ferments of other varieties is slight.

[2] C. Fränkel has proposed the name "lysogenic material" for that which acts as a bactericide.

[3] If one draws 0.2 c.c. in a capillary tube and divides the filled length of tube into twenty parts, then each part represents 0.01 c.c.

R. Pfeiffer and Marx (C. B. xxiii, 858) have shown the places of origin of the bactericidal bodies to be the spleen, and also the bone-marrow and lymph-glands, which possess specific bactericidal action much earlier than the blood. Rath (C. B. xxv, 549) could not make the same demonstration regarding agglutinin.

According to Max Gruber and Bordet, the action under 1 and 2 does not differ in principle. Their extremely simple theory, recently confirmed in the essential points by Trumpp (A. H. xxxiii, 70), is as follows:

In immune serum substances are present which cause the bacterial cells (especially their membranes) to swell, thus, without killing them, interfering with their motion and causing them to stick together.[1] In this weakened condition of the bacteria, the alexins of the body act as powerful bactericides. According to Gruber and Trumpp, also, the bactericidal action depends upon the combined effects of the agglutinin and the alexin. Trumpp proves this view by showing that also in vitro bacteria which are swollen, paralyzed, and clumped under the action of immune serum are killed by contact with the fresh serum of healthy animals. Also, Landsteiner's investigations are in accord with this (C. B. xxiii, 847).

While this sounds so exceedingly simple, still there are a series of observations which speak in favor of the view of Pfeiffer that there is an essential difference between the agglutinating and the specific bactericidal materials.

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[1] According to Paltauf and Nicolle, the agglutination is to be explained by a cementing over of the bacteria by a precipitate which is produced by the serum. (Compare Kraus and Seng, Wien. klin. Wochenschr., 1899, I.)

1. There are sera which in definite dilutions no longer agglutinate, but yet act as bactericides in the peritoneal cavity (R. Pfeiffer, Deutsch. med. Wochenschr., 1898, No. 31, 489).

2. Often an immune serum, from which all agglutinins have been abstracted by long luxuriant growth of the inoculated bacteria, which after sixteen hours are no longer paralyzed (which, therefore, is devoid of all agglutination), is still active in the peritoneal cavity.

The observations may, however, be in part explained by the discovery of Emmerich and Löw, that in the abdominal cavity the action of immune sera is very much increased by the lack of oxygen (see p. 110).

The action of agglutinin and specific bactericidal substances is, like that of antitoxin, in a great measure specific. For the bactericidal action R. Pfeiffer has maintained absolute specificity; also other authors, as Dunbar, Sobernheim, Löffler, and Abel, arrived at results that speak very much in favor of specificity (compare typhoid, cholera, etc.).

The *agglutination* phenomenon has been studied by very many investigators, and the standpoint taken by its discoverers has been confirmed as entirely correct. The action of immune sera is strongest upon the variety against which the immunity has been produced; less, but similar, against related varieties (only in high concentration); and fails with varieties that are not related.

Thus, for example, a serum from an animal which was immunized against the Bact. typhi was active in a dilution of $\frac{1}{800}$ upon Bact. typhi, and upon Bact. coli at $\frac{1}{40}$.

It is evident that this property can be of diagnostic value.

1. If we have serum from an animal which is immunized against true Bact. typhi, then it is employed to identify doubtful bacteria as typhoid bacteria, if the serum dilution of $\frac{1}{50}$ acts distinctly upon the bacteria to be diagnosticated, but not upon related bacteria; for example, Bact. coli.

2. If one has undoubted typhoid bacteria, one can as-

certain whether a man has had (Gruber and Durham) or still has (Vidal) typhoid fever, if it is demonstrated that the serum from a blood specimen in a dilution of $\frac{1}{50}$ causes marked agglutination of true typhoid bacilli, while it is without effect upon closely related organisms (Bact. coli). (For further details see special part.)

In conclusion, we may say that the essential separation of immunity into antitoxic and bactericidal appears to-day to be entirely warranted, but that in a series of cases it is established that not infrequently antitoxic and bactericidal immunity are both present. Brief reference was made above to the fact that strong diphtheria antitoxin has also some bactericidal action (van de Velde). Wassermann found that an animal protected against pyocyaneum poison also tolerates the virulent Bact. pyocyaneum in large doses, and other similar experiences are contained in the literature (compare under Cholera).

APPENDIX.

According to investigations by Emmerich and Löw which have just appeared (end of May, 1899) (Z. H. xxxi, 1), the whole doctrine of the bactericidal action of the body fluids and the immunity depending thereon appears in a surprisingly altered light.

In every old culture of bacteria, according to the authors, there are found bacteriolytic, remarkably heat-resisting enzymes—*i. e.*, ferments, which are able to dissolve and kill bacteria, especially old cells. Agglutination is only the first stage of the solution and depends, as Gruber held, upon a swelling of the external membrane. Thus, in old cultures there is always a sort of agglutination, and then a dying out of the bacterial cells occurs. The enzymes are usually only slightly specific; the pyocyaneum enzyme (pyocyanase) is, for example, active against anthrax. They operate much better if oxygen is excluded than in its presence. Also, certain bacterial poisons—for example, diphtheria toxins—are destroyed by the pyocyaneum enzyme.

If an old culture or its "metabolic products" are introduced into the body of animals, within them there occurs a union of the zymase with the body albumin—**immunproteïdin** (Emmerich). These immunproteïdins have the same solvent action upon bacteria as the bacteriolytic enzymes, but are more durable and, above all, more capable of persisting in the blood. At least in some infectious diseases the immunproteïdins can be produced synthetically in vitro instead of in the animal body, and thus, according to Emmerich and Löw, materials may be produced rapidly and cheaply which possess very high immunizing power. The immunproteïdins operate also much more strongly anaerobically than aerobically. The difference between the Gruber-Durham reaction (agglutination without death) and the R. Pfeiffer reaction (death in the abdominal cavity) is essentially dependent upon the following: In the peritoneal cavity a scarcity of oxygen prevails and the peristalsis mechanically disturbs the agglutination; also, Emmerich and Löw find the bactericidal action of normal blood to be dependent upon enzymes.

This mass of observations, which are most worthy of notice, is not to be overlooked to-day, although there has been no opportunity for substantiating them. If they prove true, they render an essential revision of the whole question of immunity necessary.

Summarized presentations regarding immunity or of the greater part of the subject are : Buchner, H., Schutzimpfung, etc., in "Handbuch der Therapie," Jena, 1897. Metschnikoff, "Immunität," Jena, 1897. Trumpp, A. H. XXXIII, 70. Dieudonné, "Experimentelle und kritische Beiträge zur Kenntnis deragglutinieren den Stoffe, etc." Habilitationsschrift. Würzburg, 1898. Dieudonné, "Schutzimpfung und Serumtherapie." Leipzig, zweite Aufl., 1899.

PART II.

SPECIAL BACTERIOLOGY.

A. Introduction to the Classification of Fission-fungi.

I. The Fundamental Ideas of Botanical Classification Applied to Fission-fungi.

All individual plants which upon careful examination are alike and transmit their characteristics to their descendants are designated as representatives of a botanical **variety** (**species**).

The nomenclature of the animal and vegetable kingdoms employed at present is founded upon the assumption that a very definite number of varieties of plants (species) are present upon our planet which can certainly be distinguished from each other by characteristics visible with more or less ease, and which, through propagation, reproduce themselves unaltered in all essential characteristics. A number of such species possess certain common characteristics and thus exhibit a certain close relationship,—these species are placed together in a **genus.** As genus characteristics it is only allowable in general to select actual characteristics, usually those concerning the structure of the organs of reproduction. Some genera consist of single species, others include hundreds. A group of genera forms a **family.**

In certain groups of the vegetable kingdom the actual circumstances suit this scheme very well. The individuals can be divided easily into a number of sharply characteristic varieties, not connected by any transition; a number of varieties group themselves naturally into a genus, and the genera constitute a natural, sharply defined family.

The conditions are nearly so in the case of the German malvaceæ. The family is sharply characterized; it consists of four genera, and each genus includes from one to seven species, which are sharply differentiated from each other. Such groups afford pleasure to the classifier.

It is entirely different with other groups. The family rosaceæ possesses very sharply differentiated genera, but in three of these (rubus, potentilla, rosa) the multiplicity of species is so great that scarcely two classifiers, in the endeavor to bring order out of chaos, arrive at the same classification. Essentially two exactly opposite methods exist for the solution of this problem. According to the first, one distinguishes every form which differs in any way whatever by a name (consequently, for example, every individual rose-bush!) and then arranges the countless forms thus obtained in the most natural manner possible. Or—and this is to-day generally preferred—a number of the most striking and widely distributed forms are selected as species, and the others are grouped as subspecies, forms, varieties, and transitional forms of these main species.

A strict classification of bacteria appears more difficult than that of any other group in the vegetable kingdom for the following reasons:

1. Bacteria, because of their minuteness and simple structure, possess very few morphologic characteristics suitable for classification.

2. The description of the individual varieties of bacteria represented in the literature has been absolutely insufficient; even recently there has been much sinning in this direction.

3. There are a great many rarely described varieties of bacteria, which can no longer be obtained in culture, with which, therefore, there is no possibility of comparison with an apparently new variety.

4. Quite a number of those describing "new" varieties have taken no trouble to look over the contributions of their predecessors, but this, to be sure, is often excusable because of the conditions represented under 2 and 3.

Still greater difficulties in the proper definition of species among bacteria lie in the *extremely great variability of bacteria*, so often referred to in the general part. Cohn and Koch could easily show that Nägeli, who had first asserted this in a broad sense, was partially led to this conclusion by inefficient methods. But also Cohn's doctrine of the constancy of species, which for a long time was most strongly advocated by Koch and his pupils, is not to-day tenable in the old sense, for continued and always more penetrating investigation has demonstrated that **almost all the properties of a well-defined species are exceedingly variable.** For example, we have learned that upon various nutrient media the microscopic forms vary throughout a wide range; that dwarf forms occur; that the liquefaction of gelatin (p. 61) and formation of pigment (p. 69), clouding of bouillon, pellicle and sediment production, ability to produce fermentation (p. 86) and pathogenic effects (p. 94) are exceedingly variable quantities, which can vary from a maximum to *nil;* even the ability of forming spores (p. 26) and, apparently, the production of flagella and spontaneous motility (p. 24) are properties that may be lost, although rarely. This means that bacteria vary as remarkably as other known plants, somewhat similarly to many cultivated plants.

For many of these variations one may recognize the cause in the influence of the nutrient medium, and speak of them as adaptations to changed conditions of life, as variations from **external causes.** Other observations, of which we related a great many in the first edition (the origin of organisms obtained upon plating a culture, which are entirely different as regards liquefaction and color, while the original culture had for many generations appeared pure), can properly be explained as dependent upon **internal causes.**

While we may deplore these facts from a didactic standpoint, since they make the teaching and learning of bacteriologic science much more difficult, and not rarely also made the solution of a concrete problem by the expert impossible, still we must not overlook them if we would advance scientific bacteriology. It is possible that the hope of those may be realized who expect that new investigations may disclose hitherto hidden diagnostic aids, which, consequently, when applied may disclose the longed-for constancy and sharp definition of species. Unfortunately, we hold the fulfilment of this hope most improbable, and look for the simplification of our subject through approaching the question from a different point of view and by an improved nomenclature.

In every species of bacterium which is closely studied, there are found closely related forms that not rarely represent to the unprejudiced unbroken links to other species. I will recall only the discoveries which have been made regarding the streptococci, the colon group, the diphtheria organisms, and the relatives of the cause of tuberculosis, which so long stood almost entirely isolated.

With this condition of things I have sought to apply to bacteria, with the greatest possible care, the principles which have been found satisfactory with the pleomorphic phanerogams, with which I have worked for years. With the principal varieties, which were completely described, we have grouped related varieties without assigning to the latter the rank of varieties. We omit this, because we must have made changes in the nomenclature, but especially *because also the principal varieties are often separated from each other by characteristics that would scarcely be considered as sufficient for the characterization of varieties in the botany of higher plants.* It is naturally almost impossible to state exactly the grade of relationship between closely standing varieties, and it often becomes a matter of taste whether one states "identical with the preceding variety" or "very closely related," etc. We certainly believe it belongs to the future to convert varieties of bacteria into others, in a manner scarcely to be imagined to-day. The forms of the Micrococcus pyogenes are convertible into each other; the Bacterium pyocyaneum and Bacterium fluorescens can, indeed, almost certainly be converted into each other; and similar statements regarding typhus and coli, diphtheria and pseudodiphtheria, etc., are always still looked upon with skepticism, but the possibility, yes even the probability, can scarcely be contested any more.

In spite of all the things which make a rational division and classification of bacteria more than ever difficult, we take the stand that it is absolutely essential to strive after it, and that also for medical men the division of bacteria into pathogenic and non-pathogenic, etc., as is still always done in text-books, has failed absolutely. We can understand and know the pathogenic varieties only if we study simultaneously the non-pathogenic, from which the former have once originated and still always originate[1] (see Pest).

The doctrine of the absolute constancy of bacteria, which for ten years was almost a dogma, is now scarcely at all seriously advocated.

II. The Nomenclature of Bacteria.

The nomenclature at present employed in bacteriologic works written by medical men is characterized by a limitless arbitrariness and inconsistency. Since these nomenclators often possess absolutely no sentiment for their arbitrariness, and the simple rules of scientific nomenclature are often entirely unknown to them, I allow myself to set down, as briefly as possible, the *most essential* rules, which are, by international agreement, accepted by all educated peoples, especially as they bear upon bacteriology.

1. Every plant and also every fission-fungus belongs to a species, every species to a genus, every genus to a family.

2. Following the precedent of Linné, every vegetable or animal organism, therefore every variety of bacterium, should have *two* Latin names: the *first* designating the genus to which the concerned organism belongs, which name is a *substantive;* the *second* indicating the variety (species), and being an *adjective* (not *two*) or the genitive of a substantive, only rarely a substantive in the nominative case. Thus, in the genus bacillus belong the species Bac. subtilis (hay bacillus), also the species Bac. anthracis (anthrax bacillus), and Bac. megatherium.

3. Genera must only be founded upon important morphologic characteristics; so-called "biologic genera," such as photobacterium for all light-emitting bacteria, pyobacterium for rods causing suppuration, etc., are only calculated to produce confusion.

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[1] If the pathologist may, perhaps, say that the pathogenic bacteria alone interest him, such a statement—as I have often heard—from the mouth of a hygienist is almost beyond understanding.

4. As designations for species many authors have used, instead of one adjective or substantive, a plurality of adjectives, evidently with the object of furnishing a description through the name: Bac. rosettaceus metalloides, Staphylococcus pyogenes aureus, Bacillus pyogenes fœtidus, Bacillus mesentericus panis viscosi I and II. This effort can be understood, but it has been *abandoned as entirely impractical* by all descriptive naturalists since Linné. The name of the species should indicate only the variety *unequivocally;* the characterization belongs to the description. It does no harm if two or more organisms possess names that mean the same, if they do not sound alike. Besides, a Micrococcus albus, also a Micr. niveus, albissimus, candicans, and purus are entirely right; the description must give more exactly the kind of differences existing between these white cocci.

5. Improperly (*i. e.*, contrary to the binomial rule) formed names may be replaced. We have done this with the greatest consideration for the existing name whenever possible.[1] We have not changed names like Bac. acidi lactici, because acidum lacticum represents a *single* idea, and names like Sempervivum Reginæ Amaliæ, Pedicularis Friderici Augusti, Trigonella Fœnum græcum, Pedicularis Sceptrum carolinum have remained, although certainly not convenient, still uncontested. Varieties which we have not studied more closely or which in our opinion should be suppressed, have not been renamed; on the contrary, Mez has conducted this renaming in the widest extent in a most acceptable manner.

6. If names are properly formed in the binomial manner and correctly published, then they must not be changed by the author himself, much less by others, even if subsequently another name appears better. Also, the reason that the name is philologically incorrect or not beautiful gives no occasion for change. Even if, for example, it was

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[1] We regret that we had to do this also in the case of a number of convenient and very familiar names; for example, those of Flügge. Unfortunately, also, Kruse has formed a large number of new names contrary to rule. Our names have the priority over his, because published about two months earlier, but they are to be preferred besides, in so far as Kruse's are formed contrary to rule.

literally more correct to call the genera which we call "mycobacterium" "tuberculomyces," such a proposition is absolutely unallowable. Renaming is only required if the name given was employed earlier with another signification. Thus, Cohn founded upon a certain organism the new genus streptothrix, without knowing that Corda, about thirty years previously, had conferred this name upon a fungus that is totally different from his. His new variety must, therefore, receive a new genus name, which he who first observed Cohn's oversight is justified in establishing.

7. It happens that an author differs from his predecessor regarding the bounds of the genera, that therefore he transfers a species from one genus into another, pre-existing or newly formed by himself. This is permissible; *still, the designation of the species must not be changed.* So we had the right, when we broke up the very large genus bacillus, following the suggestion of Hüppe, into the two genera, bacillus and bacterium, to rename a number of varieties (for example, Bacillus pyocyaneus being renamed Bacterium pyocyaneum), but we did not have a right (however much the name pyocyaneum was disliked) to rename the organism Bacterium cœruleo-viride or Bacterium Gessardi or anything else.

8. The author who names a genus places his name after it. We speak of the Bacillus Cohn, and mean the genus bacillus as Cohn established it; of the Vibrio Ehrenberg emend. Löffler, and mean the genus vibrio as established by Ehrenberg and afterward more accurately described by Löffler.

9. Whoever discovers a new species or names one not previously named *lege artis,* gives it a genus and a species name, and places his name after the latter. Flügge, who first named a large number of bacteria, gave, for example, the name Bacillus pyocyaneus Flügge to the long-known cause of bluish-green suppuration.

10. When one places a species in a new genus he puts his own name after the new name, thus, Bacterium pyocyaneum Lehmann and Neumann, but it is always to be recommended to add, in parentheses, the name of the author who first named the species. Therefore we always write,

where it does not become too cumbersome (in titles, etc.), Bacterium pyocyaneum (Flügge) Lehmann and Neumann.

While we desire that all names which express the systematic position of the variety of bacterium shall conform to the general rules of nomenclature, still we believe that names currently used in bacteriologic literature, as gonococcus, pneumococcus, staphylococcus, tubercle bacillus, diphtheria bacillus, can be still used, but as so-called *ordinary names.* Thus also the strictest botanist, if not speaking in a strictly systematic sense, often speaks of the oak instead of quercus, and strawberry instead of fragaria. We must, however, strictly avoid smuggling into the literature as names of genera such names as gonococcus, etc.

III. The Formation of the Families and Genera of Fission-fungi.

The **families** of the fission-fungi are given fairly uniformly by the more recent investigators. Here a better division does not seem possible at present; on the contrary, regarding the **genera,** the comprehension is most variable. The simplest and most natural division is that of Flügge (retained by Kruse in Flügge, third edition), which so properly includes the genera micrococcus (streptococcus), sarcina, bacillus, and spirillum, but without rejecting energetically such genera as staphylococcus, or separating the causes of diphtheria and tuberculosis. A more copious selection of genera is made by Hüppe, still more by Migula, and the most extensive by A. Fischer. After mature deliberation we have followed Flügge most closely as to the coccaceæ and bacteriaceæ, on the other hand, the works of Löffler and Migula as to the spirillaceæ.

I. Family Coccaceæ Zopf, emend. Migula. Spherical Bacteria.

Cells, when free, are perfectly globular;[1] division in one, two, or three directions of space, in which each spherical cell divides into halves, quarters, or eighths of a sphere, which again grow out into perfect spheres. Endospores and flagella very rare. Before division the cells may be one and a half times as long as broad, faint staining then revealing an unstained line of division.

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[1] Unfortunately this applies, only imperfectly to the Strept. lanolatus and Micrococcus gonorrhœæ.

1. The cells divide (almost) only in one direction of space at right angles to the direction of growth, so that if the products of division remain attached, they form (especially in bouillon) shorter or longer **rosary-like chains,** the chains often consisting of distinct pairs of cocci. Under certain circumstances there are only (or largely) pairs of cocci instead of chains. **Streptococcus** Billroth.[1]

2. The cells regularly *divide, at least on the most suitable nutrient medium* (hay decoction), in three directions of space,[2] and remain united in larger or smaller cubical family groups. **Sarcina** Goodsir.

3. The cells divide irregularly in various directions, so that there occur single cocci, single groups of from two to four cells, and, finally and preponderantly, irregular grouped bunches. Here belong all forms that do not appear as undoubted streptococci or sarcinæ. **Micrococcus** Cohn.

The recognition of these three genera of cocci is largely artificial, and there occur perfect transitions.

The genus **Staphylococcus** Ogston has no *botanical* rights, for the property of forming "grape-like" clusters is possessed at times by all varieties described to-day as micrococci. The name staphylococcus does not primarily designate any "new" genus. Ogston found (microscopically) two forms of micrococci in pus (without cultivating them), grape cocci and chain cocci, and designated them by the well-chosen names of Staphylococcus and Streptococcus (Billroth). Rosenbach later cultivated the varieties which Ogston had seen, and gave the name Staphylococcus to the bunched cocci, which we may to-day employ as the *ordinary name* for species of micrococci causing suppuration, and which we will use, but it must be dropped from the botanical classification.

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[1] Here belongs **Leuconostoc** Cienc., which is only a streptococcus with at times enormously thick capsules (see below). Also part of the "diplococci" are naturally included here.

[2] The varieties which, by division in two planes at right angles to each other, form flat groups, and which are described by authors as **pediococcus, merista, merismopedia,** we leave among the micrococci. Since even the "genus" *Sarcina* is separated with difficulty, we do not recognize the need for the genera planococcus and planosarcina of Migula, which are founded upon one or two flagellated varieties, especially as the formation of flagella varies (see below).

II. Family Bacteriaceæ Zopf, emend. Migula (Bacillaceæ A. Fischer). Rod Bacteria.

Cells at least one and a half but usually from two to six times as long as broad, straight or somewhat bent in one plane only, never spiral,[1] at times forming long true or apparent threads. Division (almost) always is at right angles to the long axis, after elongation of the rod; with or without flagella; with or without endospores. The varieties in which spores are wanting sometimes form arthrospores, according to many authors. Yet it is not possible to turn these "arthrospores" to account diagnostically, and they are entirely denied by many investigators.

1. Without endogenous spores, alleged to often have arthrospores. Rods usually less than 0.8 to 1 μ thick. **Bacterium**[2] Cohn, emend. Hüppe.

2. With endospores. Rods often more than 1 μ thick. **Bacillus** Cohn, emend. Hüppe.

Cohn in his classification laid more value upon growth into long threads (which, according to him, is characteristic of bacilli) than upon the property of spore-formation; he, however, often emphasizes the fact that most bacilli produce endogenous spores.

The fact that, through certain injurious influences, spore-formation may be lost is no valid objection to the classification, since in most cases also typical bacilli without spores are recognizable or may be conjectured. It is more unfortunate that there appear to be varieties of bacilli which at least are related to varieties in which spores never form; for example, Bacillus erythrosporus. There always appears to us to be less possible objection to the method of division adopted by us than to the other.

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[1] Unfortunately it must be said that "never spiral" is really untrue, since, for example, in the case of anthrax, Bac. Zopfii, etc., tuft-like loops occur that cannot possibly be in one plane.

[2] *Here* belongs the genus Proteus Hauser.

Critical Remarks Regarding Other Classifications of the Bacteriaceæ.

The following subdivision of the genus bacillus appears to us of little value:

Spore centrally located without a bulging of the vegetative cell. **Bacillus** in a strict sense.

Spore centrally located with bulging of the vegetative cell. **Clostridium** Prazmowski.

Spore located at the pole without a bulging of the balance of the vegetative cell. **Paraplectrum** A. Fischer.

There occur various transitions in the same species, for example, the Bac. œdematis maligni, and even, according to recent investigators, all anaerobes sometimes present clostridium, sometimes paraplectrum forms.

In the effort to build a genus classification upon the flagella, Migula[1] has arrived at the following often unnatural classification, in the more extensive application of which new complications are to be feared:

1. Cells without organs of locomotion, often with endospores. **Bacterium** Cohn, emend. Migula.
2. Cells with motile organs distributed over the whole body, often with endospores. **Bacillus** Cohn, emend. Migula.
3. Cells with polar organs of locomotion, endospore formation more rare. **Pseudomonas** Migula.

Thus in one genus are located Bac. anthracis, Bact. cuniculicida, and Streptococcus lanceolatus (!); in another, Bact. typhi and Bac. subtilis. This is contrary to all natural relationship!

The classification of the bacteriaceæ by A. Fischer is logically constructed and clearly stated. He divides the bacteriaceæ into not less than four genera without and twelve with spores, which are differentiated by the number and location of the flagella and also according to the form of the rods containing spores. Because of the great variability of these properties, this too schematic classification has won few friends. Many varieties can as well be placed in one genus as another. We desist, therefore, from giving this method of classification.

III. Family Spirillaceæ Migula. Screw Bacteria.

Vegetative bodies are unicellular, sinuously or spirally curved and twisted, more or less elongated; division always at right angles to long axis; cells often united in short chains of a few links, very often in pairs; usually actively motile because of flagella located at the ends. Endospores known in only two varieties.

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[1] Even if Migula desired to classify the bacteriaceæ according to motility, the old names of Davaine—**bacterium** for motile and **bacteridium** for non-motile varieties—certainly demanded rehabilitation.

1. Cells short, slightly bent, rigid, comma-like, sometimes attached in a screw-like manner, always one (exceptionally two) flagellum at the end. According to Hüppe, they possess arthrospores. **Vibrio**[1] O. F. Müller, emend. Löffler.

2. Cells long, spirally bent, like a corkscrew, rigid, usually with a polar bunch of flagella formed of many long principal and several short accessory ones. In the Spir. sputigenum Miller the bunch of flagella is not at the end, but on the side. **Spirillum**[2] Ehrenb., emend. Löffler.

3. Cells consist of flexible, long, spiral, coiling threads. Flagella unknown. Locomotion by means of an undulating membrane is suspected. **Spirochæte** Ehrenb.

In a strict sense the causes of glanders, diphtheria, leprosy, and actinomycosis do not belong among the fission-fungi. It is generally acknowledged to-day that they must either be designated as fission-fungi, which form a connecting-link to the higher fungi (hyphomycetes), or candidly as low hyphomycetes,[1] as was done for the first time in the first edition of this book, in 1896. Kruse has placed the **actinomyces,** together with its nearest relatives, in a family of hyphomycetes, "streptotricheæ," while he still speaks of a Bacillus tuberculosis, etc. Recently, Lachner-Sandoval has introduced the name actinomycetes to designate the group of "fission-fungi closely related to the hyphomycetes" (as we had designated them in the first edition), and until we have something better it answers for practical purposes.

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[1] Migula, with Schröter, called the group which is now almost universally designated as vibrio, **microspira**—a designation that is unnecessary if we accept the definition of vibrio suggested by Löffler. Moreover, Schröter's definition of spirillum and microspira does **not** suit the known properties of the varieties therein included. For the few non-motile (without flagella) rigid vibriones Migula has introduced the name **Spirosoma** Migula.

[2] Here belong such forms as the **Spirillum endoparagogicum** Sor., described by Sorokin and which he once found in a hollow tree in Kasan. This remarkable typically spiral-shaped organism formed typical endospores, which germinate while still within the spirillum, and so offer characteristic pictures (C. B. I, 466). The organism appears to connect the spirillaceæ and the bacilli. According to Prazmowski, the **Vibrio rugula** possesses a spore causing swelling of the end where it is located. Spore-formation has not been described in other vibriones. We know nothing regarding the flagella of this vibrio rugula, which resembles the Bac. œdematis maligni. Moreover, Zettnow expressly contradicts the idea that the vibrio rugula forms spores.

SUPPLEMENT I.

Actinomycetes (Lachner-Sandoval).

Delicately threaded organisms, free of chlorophyll, with true branching, in part very abundantly ramifying mycelium, partly with the formation of conidia. Young cultures often present only unbranched rods resembling bacteria, which can in no way be differentiated from ordinary fission-fungi. According to many authors there is a tendency to the formation of clubs or knobs at the ends of the threads.

1. Microscopic: Slender often somewhat bent rods, often with a tendency to a clubbed swelling of the ends, branches rarely observed in young cultures, easily broken off, and often difficult to find also in old cultures. Always non-motile; never conidia.

a. Rods stain interruptedly (striped) with weak staining-solutions, since the organism is composed of parts with different staining properties. Not stained by the method for the tubercle bacillus. Clubbed, wedge-shaped, and pointed rods frequent. **Corynebacterium** L. and N.

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[1] As **hyphomycetes** there have been designated for a long time in botany a large number of threaded fungi, of which nothing is known except threads and non-sexual spores that are upon threads or special carriers. The group is constantly growing smaller, as many earlier "hyphomycetes" have become known as members of the sharply characterized groups of fungi (ascomycetes, zygomycetes, basidiomycetes). The actinomycetes appear to form an entire natural group of the "hyphomycetes."

β. Rods stain with usual staining-solutions with difficulty or generally not at all. Stain by the tubercle bacillus method, *i. e.*, it is acid resisting. Clubbed swelling of the ends in cultures rare, in tissues somewhat more often. **Mycobacterium** L. and N.[1]

2. Mycelial threads, long, thin, extended, or winding, without dividing partitions, with delicate sheaths and true branches. Many species separate from the air-hyphæ rows of short spores (conidia), which, whitish and mold-like, project upward above the solid nutrient substratum; in connection with other species, conidia-formation is unknown. Not stained by tubercle bacillus method. Motility sometimes absent, sometimes present. Almost all varieties emit a musty odor. **Actinomyces** Harz.

We have determined to follow the example of Gasparini and designate this genus as **actinomyces.** **Streptothrix,** as these varieties, together with others, are called by Kruse, is a name given by Corda in 1839 to a certain mold-like organism which Cohn, because of an oversight, in 1875 introduced a second time into the literature. **Cladothrix,** which many authors to-day call these varieties (compare Günther), is the designation for an entirely different pseudodichotomous plant (see Supplement II). In the first edition we accepted, with Sauvageau and Radais, the old designation of Wallroth, **oospora,** but since Lachner-Sandoval (Dissert. Strassburg, 1898) has convinced us that the true oospora varieties are much larger although similarly constructed organisms, we also, with this author, consider the name **actinomyces** (Harz) at present the most correct.

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[1] Since we proposed this name in the first edition, we have seen that Metschnikoff (Virchow's Archiv, 113, p. 70, 1888), who first recognized the peculiar position of the tubercle bacillus as opposed to the other then known bacteria, in a work "Regarding the Phagocytic Rôle of the Tubercular Giant Cell," has said: "If one considers that in the perfected stage the tubercle bacteria have grown into (although short) threads, and also differ from other analogous forms (except the lepra bacteria) in the possession of a very dense envelope, then perhaps it will be easier to accept the designation **Sclerothrix** for the genus, and **Sclerothrix Kochii** for the species of the tubercle bacterium." We should have immediately accepted these names if we had known of them, but believe that according to the rules of botanical nomenclature our names should now stand, since Metschnikoff only made a conditional proposal, did not accurately define his new genus, and never ade any use of the new name himself, while we have ourselves ady established a name.

Some varieties of wide practical importance, closely related to bacteria, but reminding one very strongly of true algi (oscillaria), have been included under Supplement II.

If we cast a glance over this system, we can not deny that the families and genera are often connected by transition varieties; we recall only the following: The border between the coccaceæ and bacteriaceæ is obliterated by oval and lance-formed (!) cocci and certain extremely short bacilli (compare, in the special part, Micr. melitensis, Bacterium Fraenkelii); between streptococcus and micrococcus, micrococcus and sarcina, it is often unsafe to distinguish. In the cycle of growth of many bacilli twisted forms occur; flagella and endospores occur in such various forms that it would lead to an entirely unnatural grouping if the attempt were made to found a classification that depended in part upon the flagella or endospores.

The Bacterium Fraenkelii Hashimoto, for which we are indebted to the kindness of the authors, unfortunately died before we could study it. Upon solid nutrient media the organism forms short rods with polar flagella; upon fluid media, on the contrary, it forms quite long chains of cocci and occasionally sarcina forms. Thus it connects the coccaceæ with the bacteriaceæ, as does the Micr. melitensis, and shows, as we have indicated above in other examples, that sarcina forms occur as growth forms in cocci and that the presence of flagella is also variable. (See Hashimoto, Z. H. XXXI, 85.)

B. Systematic Description of the Most Important Varieties of Fission-fungi.

INTRODUCTORY REMARKS TO THE SYSTEMATIC PART, ABBREVIATIONS, ETC.

1. We have described about eighty species as completely and exhaustively as possible, several hundred are briefly described, and many

varieties which we are not acquainted with in detail are briefly referred to in the connection where they belong.

2. The colonies, slightly magnified, are described and drawn with closed diaphragm, and so placed that the *peripheral portions* are sharply visible.

3. For the drawings and descriptions plates with a medium number of colonies, 60 to 100, were always employed. Usually the smaller colonies were selected.

4. All statements, unless otherwise qualified, regarding the growth upon gelatin apply at a temperature of 22°, upon agar at 37°.

5. When nothing particular is said regarding the color and consistency in the description of the agar streak culture, and of the surface growth in the agar stab culture, they are the same as upon the agar plate.

6. Regarding the formation of **pigment, odoriferous, gustative,** and other **metabolic products** nothing has been said unless special investigations have been made upon the same.

7. Our original purpose of treating exhaustively the **resistance** of all important varieties to injurious influences has been abandoned as being too far-reaching. This decision was also partially dependent upon the fact that the statements of authors often deviate so widely. Therefore we have restricted ourselves to making complete statements regarding some varieties (Micr. pyogenes, Strept. pyogenes, Strept. lanceolatus, Bac. anthracis, Bact. typhi, Corynebact. diphtheriæ, Mycobact. tuberculosis, Vibrio choleræ).

8. **References to the illustrations in the atlas** are always given thus: Plates with Arabic numerals, figures with Roman. Thus, 5, VIII, signifies figure VIII in Plate 5.

The introductory remarks of the separate sections, coccaceæ, bacteriaceæ, spirillaceæ, are also to be heeded.

Statement of the Terms Employed by Us in the Description of Cultures of Bacteria.

I. STAB CULTURES.

A. **Not liquefying.**

1. *Stab canal:*

(*a*) Thread-like: Uniform growth without anything especially characteristic.

(α) Smooth.

(β) Rough.

(*b*) Nodular: The stab canal is beset with larger or smaller tubercles, points or teeth.

(*c*) Hairy: The stab canal is beset with delicate longer or shorter undivided spurs, which are (α) parallel, (β) curled, (γ) matted.

(*d*) Branched: The stab canal is beset with dividing outgrowths.

(*e*) Beaded: The stab canal consists of small roundish or round connected colonies.

(*f*) Band-like: Growth as a small band, produced by making the stab canal with a loop.

2. *Surface growth:*

Here the same applies as to the non-liquefying superficial colonies in the plate.

B. **Liquefying.**

(*a*) *Fixed form of liquefaction*, if the zone of liquefaction following the stab becomes larger, but assumes substantially no other form than at the beginning.

1. Tube shaped: Slowly, weak, and small.
2. Stocking shaped: Sack-shaped, rapid, strongly, at times with scalloping of the walls.
3. Vesiculated: Bubbles are formed and confined in the depth.

(*b*) *Variable form of liquefaction.*

I. Initial stage:
  1. Saucer shaped.
  2. Funnel shaped.
  3. Flattened funnel shaped.

II. Advanced stage:
  1. Cylindrical: The liquefaction extends more above and soon reaches the glass, and then extends, with a horizontal limiting surface, downward.
  2. Funnel shaped: The liquefaction extends more uniformly from the culture. The funnel shape is preserved still in later stages. Often the second form is succeeded by the first.

II. STREAK CULTURES.

A. **Surface growth:** The same designations apply as to the surface cultures upon plates.

B. **Water of condensation.**

(*a*) Clear, with or without sediment.
(*b*) Cloudy, with poorly defined sediment.
(*c*) Pellicle on surface.

III. BOUILLON CULTURES.

A. **Fluid:**

(*a*) Clear.
(*b*) Cloudy.
(*c*) Syrupy, gelatinous.

B. **Sediment:**

(*a*) Cloudy.
(*b*) Flocculent, if upon shaking it rises as a twisted column, and can be homogeneously distributed.
(*c*) Sandy, if it lies steadily at the bottom and, upon shaking, is distributed as small fragments.

IV. POTATO CULTURES.

The same designations apply as to the streak and plate cultures.

V. PLATE CULTURES.

A. **Without liquefaction.**

(*a*) *Form:*

1. Point-like, when the dimensions are very slight.

2. Round, circular.
3. Roundish, not perfectly circular.
4. Oval.
5. Whetstone shaped, pointed at both ends.
6. Curled, coiled.

(*b*) *Elevation:*
1. Flat.
2. Veil-like.
3. Wavy.
4. Reticulated.
5. Terraced.
6. Elevated.
7. Nail-head.
8. Drop-like.
9. Corniform.

(*c*) *Optical peculiarity of surface:*
1. Moistly shining, highest degree of luster.
2. Greasy.
3. Faintly shining.
4. Dull.
5. Finely granular.
6. Transparent.
7. Iridescent, pearly.
8. Opaque.
9. Chalky.

(*d*) *Consistency:*
1. Veil-like.
2. Membranous.
3. Leathery.
4. Tenacious.
5. Slimy.
6. Cartilaginous.
7. Friable.
8. Butter-like.

(*e*) *Peculiarity of border, especially slightly magnified with microscope:*
1. Entire.
2. Rough.
3. Smooth.
4. Dentate.
5. Lobulated.
6. Scalloped.
7. Ragged.
8. Short-haired.
9. Long-haired.
10. Curly.
11. Matted.

(*f*) *Internal structure:*
1. Homogeneous (without structure).
2. In zones.
3. Radially striped.
4. Radially wrinkled.
5. Finely dotted.
6. Coarsely dotted.
7. Granular.
8. Coarsely granular.
9. Finely lobulated, mulberry-like.
10. Coarsely lobulated, scaly.
11. Irregularly spotted.
12. Grained.
13. Curly.
14. Crumbly.
15. Matted.

B. With liquefaction.

(*a*) *Form:*
1. Saucer-shaped depression.
2. Pocket-shaped depression.

(*b*) *Appearance:*
1. Liquefied medium clear—
    - (α) With compact original colony.
    - (β) With original colony disintegrating.
2. Diffusely cloudy.

Special Introductory Remarks Concerning the Coccaceæ. Spherical Bacteria.

1. Since almost *all* the varieties presented, with the exception of the Micr. gonorrhœæ, stain with the ordinary anilin dyes and by Gram's method, we usually state nothing regarding the staining properties, except when they *can not be stained by Gram's method.*

2. *When no mention is made of flagella and spores, they are absent.*

3. No mention is made of the *intense stain* with watery solutions of anilin dyes, which occurs with all varieties, and a similar statement would have to be always repeated. It is strongly recommended, when it is desirable to obtain the cement substance between the bacterial cells unstained (capsules), to employ a *dilute aqueous solution of anilin dyes,* or after staining with stronger solutions to employ dilute acetic acid as a decolorizing agent, or to use Gram's method. This is obligatory in the case of sarcinæ and diplococci in order to render the line of fission in dividing cocci visible, etc. (An exception is the gonococcus.)

4. Since all varieties of the genus micrococcus not infrequently occur as diplococci, tetrads, and short chains, we have only said anything regarding *the grouping when there is something special to notice.*

5. For an exhaustive discussion upon suppuration and the part played by micro-organisms in the same, see Kurt Müller, C. B. xv, 735, and Poliakoff, C. B. xviii, 33.

FAMILY I.—COCCACEAE. SPHERICAL BACTERIA.

Family diagnosis and genera scheme, see page 122.

1. Streptococcus (Billroth).

The cells divide only in one direction of space at right angles to the direction of growth, so that if the multiplying cells remain attached to each other, shorter or longer rosary-like chains are formed. Often the chain appears to be be built up from distinct pairs. Chains are formed with most constancy in bouillon; upon gelatin and agar, as also in the animal body, very often no chains occur. It is therefore always desirable to prepare *bouillon cultures of any variety in which there is a suspicion of a streptococcus* before arriving at any conclusion. It is not unusual to find single members in a streptococcus chain of somewhat larger dimensions than the rest, but otherwise exactly resembling the other members of the chain. It is, at least, so far not certain that the cells contain arthrospores, as many authors believe.

Key to the Recognition of the Most Important Varieties of Streptococci.

I. Strings of cocci upon all nutrient media (also upon those containing grape- and cane-sugar), *without thick capsules;* at most, with delicate capsules.

(A) Do not grow as a yellow "creamy layer" upon sheep- and calf-serum, and microscopically are without wide capsules.

(*α*) Cocci spherical or, when dividing, hemispherical, capsules almost always absent.

1. Gelatin liquefied slowly or not at all. Cells 0.6 μ to 1 μ; long or short chains; often thrive better anaerobically; slight growth on all nutrient media; pathogenic or non-pathogenic. **Strept. pyogenes** Rosenbach,[1] page 135.
2. Gelatin rapidly liquefied in tube form; cells very minute (0.2 μ to 0.4 μ); forms long chains, and grows poorly upon potato, agar, and serum. According to Escherich ("Die Darmbakterien des Säuglings," Stuttgart, 1886, p. 77), it is constantly present in the feces of carnivora. Not pathogenic for guinea-pigs. Strept. coli gracilis Escherich. **Strept. gracilis** (Escherich) Lehm. and Neum.

(*β*) Cocci more or less lance-shaped, capsules usually absent in artificial media, but never in animal body. Upon gelatin, poor growth and no liquefaction. **Strept. lanceolatus** Gamaleia,[2] page 143.

(B) Form a yellow creamy layer upon fluid sheep- and calf-serum. Microscopically from these nutrient media they have wide unstained capsules. **Strept. involutus** Kurth, page 149.

II. Chains of cocci upon grape- and cane-sugar nutrient media with thick gelatinous capsules, which may be ten times as thick on all sides as the chain of cocci. Upon other nutrient media it is not differentiated microscopically from Group I. **Strept. mesenterioides** Migula, page 150.

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[1] The streptococci scorn every exact method of division. That given here, while apparently a convenient and accurate scheme of division, suffers very much in the closer description of varieties from peculiarities, transition forms, etc.

[2] Compare also Strept. intracellularis (Weichselbaum) Lehm. and ·um., page 148.

Streptococcus Pyogenes (Rosenbach[1]).

(Plate 1.)

Synonyms. — Strept. erysipelatos Fehleisen, Strept. puerperalis Arloing, Strept. articulorum Flügge, Strept. pyogenes malignus Flügge, Strept. septicus Nic, Strept. scarlatinosus Klein (compare also pp. 140 and 141).

Ordinary Names.—Chain coccus, string of pearls coccus.

Most Important Literature.—Rosenbach ("Mikroorganismen bei den Wundinfektionskrankheiten des Menschen," 1884). Fehleisen ("Aetiologie des Erysipels," Berlin, 1883). v. Lingelsheim (Z. H. x, 331; xii, 308). Kurth (A. G. A. vii, 389). Behring (C. B. xii, 192). Knorr (Z. H. xiii, 1893, 427). Pasquale ("Ziegler's Beiträge," xii, 433,—extensive list of literature). Marmorek ("Wiener med. Wochenschr.," 1895, 1346). Koch and Petruschky (Z. H. xxiii, 477). Widal and Besançon (C. B. xx, 240).

Microscopic Appearance.—The characteristic chain growth presents itself especially in fluid cultures (bouillon). Upon solid nutrient media and in the animal body the chains are often very short or the arrangement extremely irregular (1, ix, x).

Upon close observation of faintly stained preparations the individuals of the chain usually consist of two hemispheres, which are connected with each other and the adjacent member of the chain by a colorless mass. More rarely definite mucoid capsules are seen about the chains (compare Babès, Z. H. xx, 412).

Staining Properties.—As usual and well by Gram's method.

Relation to Oxygen.—Facultative anaerobe, sometimes better aerobically, sometimes better anaerobically.

Requirements as to Temperature and Nutrient

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[1] Since all efforts to divide the streptococcus pyogenes into several sharply differentiated varieties must be recognized as failures, because connecting transition forms between the subvarieties occur, so we shall treat the variety as a unit, and at the end will add something regarding its forms.

Media.—Growth rather slow, best at 37°. Above 47° no growth (Arloing).

They also grow more slowly but more luxuriantly upon feebly acid nutrient media (hydrochloric acid, tartaric acid). Grow more slowly but with greater vitality at 23° than at 37°. Especially good growth occurs in exhausted cholera or pyocyaneum bouillon either after or without filtration (Turró, C. B. XVII, 865).

Gelatin Plates. — (*a*) *Natural size:* Very small, whitish, roundish, flat, rarely slightly elevated colonies, which do not grow perceptibly after a longer time (1, V). (*b*) *Magnified fifty times. Superficial:* Roundish colonies with smooth border (1, VII e), but may present also wavy, scalloped, serrated, as well as fringed and torn forms (1, VI e). Color is gray to yellowish, structure delicately punctate to finely granular, usually transparent. *Deep lying:* Roundish to whetstone-shaped, rough or smooth border, somewhat more coarsely punctate than the superficial (1, VII i; VI i).

Gelatin Stab.—*Stab:* At first thread-shaped; after a short time there appear numerous small nodules in the stab (1, II). Surface growth is like that in the gelatin plate.[1]

Gelatin Streak.—Narrow, beautiful, delicate growth along the streak, beset at the borders with little nodules.

Agar Plates.—(*a*) *Natural size:* As on gelatin plates. (*b*) *Magnified fifty times. Superficial:* Spherical colonies with delicately punctate edge, transparent, grayish-yellow, at first very delicately punctated, later (fourteen days) at times granular; frequently there is a distinct appearance of lobulation (1, VIII e). *Deep lying:* Smaller and somewhat darker (1, VIII i).

Glycerin-ascites-agar.—Colonies distinctly more luxuriant. From the periphery of the superficial colonies there often extend outward numerous shorter or longer coiling chains, so that the colony appears not unlike a young anthrax colony. Also, the granulation in the interior of the colonies is somewhat more marked than upon agar.

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[1] Liquefaction, according to German authors, is very rare. Pane saw the Strept. pyogenes from human abscesses at a temperature above 24° produce regularly liquefaction of gelatin which he had so prepared artificially that it was first melted at 30° (C. B. XVI, 228).

Agar Stab.—*Stab:* Thread-like, later sometimes granular (1, III). *Surface growth:* Very delicate growth, transparent, gray, irregular, unimportant. Atypically, the growth may be much more vigorous, with whitish-gray color and smooth wavy border (1, IV). Similar also on glycerin agar.

Agar Streak.—As on gelatin. *Water of condensation:* Clear with slight whitish deposit.

Bouillon Culture.—Varies greatly in the different forms, from diffuse cloudiness to the formation of a compact sediment with clear fluid (see p. 141).

Milk Culture.—Usually firmly coagulated in from four to twenty-four hours.

Potato Culture.—Invisible growth, at times entirely absent, rarely more luxuriant (compare p. 141).

Non-albuminous Medium.—Faint growth.

Vitality.—*In cultures* usually only a few weeks. According to Petruschky, cultures on gelatin, grown for forty-eight hours at 22°, if kept in an ice-box retain their vitality and virulence for months. The Strept. pyogenes belongs among the varieties that die quickly. Bouillon cultures, if oxygen is admitted, usually live only for weeks, but in hydrogen for months.

Resistance to Drying.—Vitality and virulence are retained several months, especially in dried *pus*.

Chemical Activities.—(*a*) *Chromogenesis:* Almost always without pigment production; cultures were grown by Kruse and Pasquale in Italy with yellowish-brown to blood-red pigment. These were highly virulent, short-chained forms obtained from cases of tuberculosis.

(*b*) No *indol*, little *sulphuretted hydrogen*.

(*c*) *Acid production from carbohydrates* in our cultures was minimal; no gas formation.

According to Sieber-Schoumoff, certain cultures (Strept. erysipelatos and Strept. scarlatinæ) produce levorotatory lactic acid, others (Strept. pyogenes) inactive lactic acid from grape- and milk-sugar. All cultures produce, besides, some volatile fatty acids, poisonous albumoses, and of gases only carbonic acid, with the exception of the form found in scarlatina, which also produces hydrogen.

Emmerling's investigations (C. B. L. IV, 342) regarding the decomposition of fibrin by streptococci under anaerobic conditions gave the remarkable result that a solution of fibrin was effected. He found succinic, acetic, propionic, normal butyric, and caproic acid, methylamin, trimethylamin, collidin, but no toxins.

(*d*) *Toxin production:* Upon albuminous nutrient media streptococci produce toxins, soluble in water and precipitated by alcohol. To collect them the cultures are killed with chloroform or filtered through porcelain. Large doses of the metabolic products cause suppuration and fever, and even death. This appears always to be only the action of protein.

Occurrence.—(*a*) *Outside the body:* In soil, canal-water, once in a well (Landmann, C. B. XIV, 431), in the air of operating rooms, etc.

(*b*) *In the healthy body:* In mouth, nasal cavities, vagina, not rarely cervix uteri; at times, moreover, in a virulent form.

(*c*) *In diseased human organism:* The streptococcus is capable of causing a large number of diseases, namely, inflammation and suppuration in all parts of the body. It causes especially often the following diseases: Erysipelas, phlegmonous abscess,[1] lymphangitis, follicular angina, bronchitis, impetigo contagiosa, cellular pneumonia (Finkler), pyemia, septicemia, and puerperal fever. More rarely, pleuritis, pericarditis, meningitis, enteritis,[2] etc., some cases of osteomyelitis, elephantiasis nostras (Sabouraud).

Recently, Escherich with his pupils has emphasized the significance of the streptococci in the diarrheas of children. The form isolated from such cases can not be imagined as a new sharply defined variety, in spite of slight deviations, but belongs in the division of the Strept. pyogenes or lanceolatus. Escherich, Th., "Ueber Streptokokkenenteritis im Säuglingsalter." Separatabdruck aus Jahrbuch f. Kinderheilkunde, N. F., Bd. XLIX, 1899.

It is found in the blood and urine rather often, either with or without symptoms of a general disease.

The following also certainly depend upon Strept. pyogenes infection: Some cases of nephritis, articular rheumatism, myelitis, and infantile paralysis. Mannaberg has found it in fourteen cases of Bright's disease (C. B. v, 93), whether as primary cause is questionable.

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[1] In phlegmons and abscesses more often the staphylococcus (Micr. genes) is present, or a mixture of both.

In the institute for infections diseases in Berlin, Beck described a of streptococcus infection (intestine, blood, viscera) that caused h in three days and presented during its course the typical picture siatic cholera (C. B. XI, 632). Compare Tavel, de Cérenville, (C. B. XVIII, 547).

The streptococcus plays an *important rôle* in diphtheria, scarlatina, and phthisis. It *accompanies* the specific cause of disease, and *markedly* influences the disease-picture, especially the course of the fever (hectic fever is streptococcus fever) (Petruschky, Z. H. XVII, 59).

(*d*) *In animals:* As the cause of similar diseases (compare, for example, Strept. equi, p. 142).

In the vaccine of cow-pox institutes it is not uncommon, but usually possesses little virulence.

Experimental Observations Regarding Pathogenic Action.—With living cultures. The virulence fluctuates greatly; even freshly isolated organisms may be very faintly virulent, and virulence for experimental animals does not prove virulence for man; with cultivation upon the ordinary nutrient media the virulence is rapidly lost. By repeated transmission through animals, a virulence which was high at first may be much intensified. Marmorek obtained cultures of such virulence that $\frac{1}{1000}$ c.mm. killed almost all, and $\frac{1}{10000}$ c.mm. some, mice when given subcutaneously—*i. e.*, quantities that contain only relatively few germs.

The virulence is well preserved, according to Marmorek, upon (1) two parts of human or horse serum and one part of bouillon; (2) one part of fluid from ascites or pleural exudate and two parts of bouillon, even after keeping two months in the incubator without transfer to fresh nutrient media.

In general the most susceptible to the streptococcus among animals are mice and rabbits; much less, dogs and rats (Pansini). Streptococci are still better tolerated by sheep and goats, and best by the horse and ass.

Knorr has ascertained the following *principal* points regarding the virulence: By repeated transmission through mice an organism is obtained which is very pathogenic for mice, but at the same time its virulence for rabbits was gradually lost. This is a strong indication that *one must not found any species upon a specific virulence.* The more virulent a form is for a variety of animal, the more certainly it kills without suppuration, the latter being caused only by feebly virulent forms.

Almost all the diseases enumerated above may be produced experimentally in animals; the result in experimental animals depends very largely upon the virulence and amount of infectious material.

Also in man streptococci have been successfully inoculated (erysipelas, phlegmon).

Immunity and Immunization.—If an animal resists an injection of the metabolic products, and has after a time recovered from the consecutive cachexia and loss of weight, then the dose may be increased and gradually a high degree of immunity be obtained. Yet the statements of Marmorek are contested, when he claims that horses and asses may thus supply a serum which cures human sepsis (Petruschky, Schenk). At any rate it has been shown, according to the investigations of Denys and his pupils (C. B. XXIV, 685), that the individual varieties of streptococci yield a serum that is active only against the particular variety employed in producing the immunity; thus also animals, in order to yield serum of therapeutic value, are to be treated with the most variable cultures possible of streptococci ("polyvalent serum"). Regarding the way in which the serum acts, compare page 97.

Special Methods for Demonstration.—Microscopic form and staining by Gram's method; agar plate in incubator; bouillon culture to obtain chains; animal investigation (mouse).

Forms and Subvarieties of the Strept. Pyogenes.

All efforts of authors to characterize sharply the forms of the Strept. pyogenes as varieties, subvarieties, or species, and to cover them with names are to be considered as failures. Countless transition forms and the enormous variability of all the properties make every classification appear insufficient. Even the separation from the Strept. lanceolatus is not always possible. Pasquale (Ziegler's Beiträge, XII, 433), Lemoine (H. R., 1896, 892), Widal and Besançon (H. R., 1896, 996), and Petruschky (H. R., 1897, 772) have all come to analogous results from their minute studies.

Behring and his pupil v. Lingelsheim arrived at the following useful[1] division:

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[1] There are found by many authors a "Strept. brevis" without

(*a*) In bouillon form short, slightly tortuous chains; bouillon cloudy; gelatin is very slightly liquefied; significant growth upon potato; growth even at 10° to 12°. Virulence usually absent. **Strept. brevis** v. Lingelsheim.

(*b*) In bouillon the streptococci form very tortuous, long chains (forty and more members), which make up a flocculent or slimy sediment, leaving the bouillon clear. Gelatin always remains solid; visible growth on potato is absent, virulence is usually great. No growth below 14° to 16°. **Strept. longus** v. Lingelsheim.

The subdivision of the Strept. longus into the following varieties (Behring) has now only a historical interest, since according to Behring's pupil, Knorr, the characteristics of these subvarieties, *upon repeated cultures*, are variable, and so the identity of these subvarieties can be demonstrated: (1) **Turbidus,** with turbid bouillon culture; (2) **viscosus,** with clear bouillon culture and delicate sediment; (3) **conglomeratus,** with clear bouillon and granular sediment. The same was also found by Kruse and Pasquale (Ziegler's Beiträge, XII, 1893, 433). Interesting but unsatisfactory is also Pasquale's attempt at a classification of streptococci (C. B. XV, 761). Also Babès came to little sharp differentiation; for him, as for us, all forms (including the Strept. lanceolatus) are connected by transition forms.

The findings of Waldvogel are interesting. Three times he obtained, after inoculation with Strept. longus (the bouillon remained clear and there was an insignificant granular sediment), from the heart's blood of the inoculated mouse an organism forming chains with from four to six members, and causing a diffuse cloudiness of bouillon. Upon potato both forms grew equally poorly. By growth in strongly alkaline bouillon the long chain form could be transformed into one producing a slight diffuse cloudiness; and by growth in almost neutral bouillon of the form producing turbidity a race was again obtained which produced no flocculi in clear fluid and formed long chains.

After such experiences more recent authors do not make a division of the Strept. pyogenes into different forms, and prefer to designate the forms described by them as Strept. pyogenes, the form being described in a few words. We also believe this to be right. Compare also Zenoni, C. B. XXI, 10, and the interesting studies of Seitz concerning

gelatin liquefaction, and a Strept. longus with slight liquefaction; also occasionally a Strept. longus with a visible and a Strept. brevis without a growth on potato. Marignac and d'Espine found Strept. brevis which formed sediments in bouillon and did not cloud it. Marbaix proved complete independence of the length of the chains and pathogenic quality.

maststreptococci (**Strept. aggregatus** Seitz; C. B. xx, 854) from the mouth, which with their very marked variability still always belong in the group of the Strept. pyogenes.

Streptococcus equi (Kitt). Drusestreptococcus (Schütz).

All the morphologic characteristics agree throughout with the Strept. pyogenes, also the pathogenic effect fluctuates as in it. Details concerning it by Cappelletti and Vivaldi (A. H. XXXIV, 1). Also in horses, as in man, streptococci cause pneumonias, the organisms resembling sometimes the Strept. pyogenes, more often the Strept. lanceolatus (compare Lignières-Alfort, C. B. XXII, 768). "Druse" (French "*gourme*") is an inflammation of the upper air passages in horses, with inflammatory disease of the adjacent lymph-glands, in which not rarely abscesses form. The differentiation between glanders and this disease is easy by microscopic examination and the positive results of inoculation of house mice (Schütz, C. B. v, 44).

Streptococcus agalactiæ (Adametz) = Strept. mastitidis sporadicæ Guill., Strept. mast. epidemicæ Guill., Galtcoccus.

Morphologically sometimes a short, sometimes a long-chained Strept. pyogenes. Cause of the "*gelbe Galt*," a sporadic or epidemic inflammation of the udder of cows and goats. The milk becomes very scanty, yellowish, beset with flocculent coagula and often gas-bubbles. The form producing long chains is more virulent than the one occurring in short chains. It is important that many cultures break up grape- and milk-sugar energetically with gas-formation, according to Nencki, especially with the formation of dextrorotatory paralactic acid and carbonic acid (no hydrogen), traces of fatty acids, and alcohol. This fermentation of the milk causes a low grade of cheese (inflated cheese). The virulence and ability to cause fermentation vary in this organism very much. (Compare Adametz, "Milchzeitung," 1893, and Zschokke, C. B. XXII, 784.)

The **Micr. acidi paralactici** Nencki (C. B. VII, 130) and **Strept. acidi lactici** Grotenfeldt ("Fortschritte der Medizin," VII, 121) appear to be closely related; the latter forms no gas and thrives especially anaerobically. Also similarly the **Micr. Sornthalii** Adametz (C. B. L. I, 465), an organism fermenting milk with intense production of gas (CO_2 and H) and causing inflation of cheese, which in its cultural behavior upon gelatin plates reminds one somewhat of the Strept pyogenes. In stab cultures the growth is somewhat more profuse. Microscopically, it is a round or oval coccus, either single or in short chains. Krönig has described varieties of anaerobic non-pathogenic streptococci, preferring acid nutrient media, which were obtained from the human vagina.

Not at all characteristic are the species also isolated from cheese by Henrici, which were not examined as to their effects upon sugar, milk, potato, and animals (A. K. B., Heft I, 1); and **Strept. tyrogenus, albidus, magnus, granulaius, pallens, pallidus,** Henrici,[1] which are only differentiated by characteristics that are not very pronounced and are still to be tested as to their constancy (more or less granulation in the plate cultures, character of cloudiness in bouillon, slightly different adaptability to aerobic and anaerobic life). The **Strept. stramineus** Henrici, which grows as a straw-yellow, shining deposit, appears to differ more strongly.

Streptococcus lanceolatus[2] (Gamaleia). (A. P., 1888, ii, 440.)

(Plate 2.)

Synonyms.—Diplococcus pneumoniæ A. Fränkel and Weichselbaum, Dipl. of sputum septicemia A. Fränkel, Meningococcus Foà, Pneumococcus Foà, Dipl. lanceolatus sive lanceolatus capsulatus Foà and Bordoni-Uffreduzzi, Bact. pneumoniæ Migula, Micr. pyogenes tenuis Rosenbach (C. B. VII, 177).

Ordinary Names.—Capsule coccus of pneumonia, pneumococcus, Fränkel's pneumonia coccus.

Literature.—Exhaustive critical studies by Kruse and Pansini (Z. H. XI, 279), Levy and Steinmetz (Arch. exp. Path., 1896, 89). Literature by Schabad (C. B. XIX, 991).

Microscopic Appearance.—Arranged usually in pairs or chains of from four to six members, roundish or—what is especially characteristic—*lancet-shaped* (2, X). When obtained from the animal body or when cultivated upon sterilized sputum and tracheal mucus, or in fluid rabbit's serum, it usually presents a significant capsule, which may be stained (p. 22, Fig. 5) (2, IX).

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[1] Here also belongs the **Strept. cinereus** Zimmermann (Bd. II, 64), obtained from tap-water, which is said to present somewhat more prominent cultures on gelatin plates.

[2] Since the name Strept. pneumoniæ is applied by Weichselbaum to a Strept. pyogenes from cases of pneumonia, it would lead to confusion if, following the rules of strictly botanical nomenclature, the Dipl. pneumoniæ was renamed simply the Strept. pneumoniæ. On the contrary, the name Strept. lanceolatus is also very old (1888), characteristic, and unmistakable.

Often single members present larger dimensions and the form of a club—*i. e.*, to a large sphere is attached a small, thin neck-piece. These are not, however, resting forms. (Compare Stolz, C. B. XXIV, 337.)

According to Kruse and Pansini and our own investigations, all transitions up to the Strept. pyogenes occur, so far as concerns the form of individuals and the structure of chains. (Compare also Binaghi, "Ueber einen Strept. capsulatus," C. B. XXII, 273.)

Relation to Oxygen.—Facultative anaerobe.

Intensity of Growth.—Grows fairly rapidly but not luxuriantly at 37°. At ordinary temperature (22°) very slowly, and more often not at all.

Gelatin Plates.—(*a*) *Natural size. Superficial:* Roundish, dim, diffusely gray, transparent colonies, which after four days have attained a diameter of from 1 mm. to 2 mm. *Deep:* Very small, roundish, whitish-gray (2, V).

(*b*) *Magnified seventy times. Superficial:* Circular or roundish colonies with almost smooth border, colorless, and delicately granular. They are often so delicate that with the narrowest diaphragm the periphery can hardly be differentiated from the surrounding medium (2, VIII, *e*). *Deep:* Round, sharply outlined, slightly more granular (2, VIII, *i*).

Gelatin Stab Culture.—*Stab:* At first thread-like, later resembling a string of pearls; growth faint. *Superficial growth:* Minimal, almost none (2, I). No liquefaction.[1]

Agar Plates.—(*a*) *Natural size:* Like gelatin plates (2, V).

(*b*) *Magnified fifty times. Superficial:* Roundish, almost even border, at times somewhat fringed, delicately punctate, a little more compact than the gelatin culture, colorless, perfectly transparent (2, VI). *Deep:* Roundish or whetstone-shaped, almost even-bordered, opaque, gray to grayish-black, more coarsely punctate than the superficial (2, VII).

Agar Stab.—*Stab:* Thread-like, whitish-gray (2, III).

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[1] MacCallum and Hastings have described a liquefying form (analogous to certain rare varieties of the Strept. pyogenes) as **Micr. zymogenes** (C. B. XXV, 384).

Surface growth: Very delicate, transparent growth, with even border, faintly glistening (2, IV).

Agar Streak.—Extremely delicate, transparent, grayish-white, faintly glistening, often not sharply outlined from the agar. Water of condensation clear, with very little whitish sediment (2, II).

Serum Culture.—Slimy, almost transparent growth.

Ascites-glycerin-agar. — More luxuriant cultures. Those lying superficially are usually even-bordered, the periphery somewhat padded, and throughout (especially in old colonies) coarsely punctated to mulberry-like. They then resemble old gonorrhea cultures or at times even very young agar cultures of the colon bacillus.

Bouillon Culture.—Short, straight chains; sediment light and not holding together (Kurth).

Milk Culture.—Milk coagulated. This property, according to Kruse and Pansini, is very rarely absent. In the milk small amounts of acid are formed.

Potato Culture.—*No growth.*

Vitality in Cultures.—Very short duration of life (often only a few days), and even a more rapid lessening of virulence. In bouillon occurs the most luxuriant growth, but it is least durable.

Resistance to Drying.—In dried blood as long as forty-five days; in dried sputum as long as one hundred and twenty to one hundred and forty days in diffuse light, and nine to twelve hours in direct sunlight. Literature, Germano, Z. H. XXVI, 66.

Chemical Activities.—Fawitzky isolated three cultures, which were able to produce a brick-red pigment (best in bouillon). (Compare Strept. pyogenes.) Filtered and devitalized unfiltered cultures contain toxins, but in relatively small amount. In other respects it is like the Strept. pyogenes.

Occurrence.—(*a*) Outside the organism: Not found.

(*b*) In healthy organisms: Often in saliva.

(*c*) In diseased human organism: One of the *most important pathogenic* varieties. In the most various inflammatory processes, especially such as attack mucous and serous membranes, also not infrequently causing suppuration. Especially frequent as the cause of croupous and catarrhal pneumonia, pleuritis, pericarditis, endocarditis, peritonitis, otitis, meningitis, conjunctivitis, and ulcus serpens corneæ. More rarely as the cause of nephritis and perinephritis, metritis, pyosalpinx, strumitis, parotitis, amygdalitis, arthritis,[1] osteomyelitis, periostitis, abscesses, and general sepsis. It may also cause erysipelas (Schürmayer, C. B. XXIII, 183). In many of these diseases the organism is found not only locally, but also in the blood. Very often other exciters of inflammation accompany and aid the Strept. lanceolatus, which is always more difficult to cultivate, so that if ordinary agar is employed for cultures, staphylococci, etc., may alone be observed. Therefore ascites-agar and similar media are to be preferred. The Strept. lanceolatus escapes from the diseased person in the milk and urine.

Regarding the participation of the Strept. lanceolatus in cerebrospinal meningitis, see under Strept. intracellularis, page 148.

Marchoux (A. P. XIII, 193) repeatedly found in soldiers, as a sequel to pneumonia, a tendency to sleep ("Schlafsucht," *maladie du sommeil*), and upon section there were changes in the cerebrospinal membranes with the Strept. lanceolatus present.

Experimental Observations Concerning Pathogenic Effects.[2]—(*a*) *In animals:* Of animals, the rabbit and mouse are especially susceptible, the rat less so, and guinea-pigs, sheep, dogs, and birds almost not at all.

The mouse dies in from twelve to twenty-four hours after subcutaneous infection of septicemia; spleen enlarged, eyelids glued together. In the blood are large numbers of diplococci. In mice pneumonia also can be produced by inhalation. Likewise in rabbits septicemia with fever and swelling of the spleen follows subcutaneous and more rapidly intravenous inoculation with strongly virulent cultures; death follows in forty-eight, twenty-four, twelve, or even five hours. Attenuated cultures cause, according to the point of inoculation, pneumonia, pleuritis, peritonitis, etc. Honl especially recommends for diagnosis and for demonstration purposes the subcutaneous injection of sputum in the rabbit's ear; death follows after two to five days, and the bacteria are found especially numerous and with typical capsules in the edematous fluid, which is obtained by incision of the doughy infiltration over the lower jaw (C. B. XXIII, 274).

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[1] Here also belongs the excitant of chronic deforming inflammation of joints, described by v. Dungern and Schneider (Münch. med. Wochenschr., 1898, No. 43, 1369).

[2] The virulence is exceedingly variable and in the usual cultures it is rapidly lost. For the preservation of the virulence of the Strept. lanceolatus during about two months it was recommended, for example, by Bordoni-Uffreduzzi to dry upon glass the blood of rabbits which the infection had killed. Foà places such blood for twenty-four hours in the incubator, and then preserves it in the cold.

(*b*) *In man:* Subcutaneous injection of from 0.1 to 0.2 c.c. of *virulent* culture in seven men was without important effect except local symptoms, some fever, and headache.

Immunity and Immunization.—Mennes, whose careful work (Z. H. XXV, 413) should be consulted in the original, has recently obtained fairly active protective serum. The action of the serum consisted in this, that it renders the leukocytes of normal animals capable of devouring the Strept. lanceolatus (phagocytosis). Encouraged by the investigations upon animals (Emmerich and Fawitzky, Foà, Klemperer), curative injections of the metabolic products and the serum of immunized animals have been tried also upon man, but so far without indisputable practical results.

Special Culture Methods.—The Strept. lanceolatus is most easily obtained by inoculating a mouse or rabbit with fresh rusty sputum from croupous pneumonia, and making cultures from the heart's blood of the dead animal upon ascites-agar plates. It is also often easily obtained from an eye with ulcus serpens corneæ by the preparation of streak or plate cultures upon ascites-agar, and placing them in the incubator.

Forms and Subvarieties of the Strept. lanceolatus.

We must frankly admit that a sharp separation of the Strept. pyogenes from the Strept. lanceolatus seems to us, as to many authors, to be impossible, if the typical form of the Strept. lanceolatus is to be determined by capsules, lancet-shaped individuals, and a tendency to form only very short chains. Many investigators who have especially studied the Strept. lanceolatus have tried to set up definite forms, which can scarcely be identified subsequently. Almost all of these divisions have consisted of somewhat differently defined varieties, as is the case with the Strept. pyogenes. (Compare Kruse and Pansini, Z. H.

XI, 279; Pansini, Virchow's Archiv, CXXII, 424; Banti, C. B. IX, 275; Foà.)

Streptococcus intracellularis. (Weichselbaum.) Lehm. and Neum.

(Plate 68, III, IV.)

Synonym.—Diplococcus intracellularis meningitidis Weichselbaum.

Literature.—Jäger (Z. H. XIX, 351); Weichselbaum ("Fortschritte der Medizin," 1887, V, 573). Recent literature is comprehensively reviewed by Kamen (C. B. XXIV, 545); in the latter place (as also in the article by Jäger) are found illustrations.

While a number of authors—for example, Bordoni-Uffreduzzi and Foà; Paniénski (C. B. XVIII, 651); Henke (C. B. XXII, 59)—have found the Strept. lanceolatus to be the cause of **cerebrospinal meningitis**, and others—for example, Bonome—have found the **Strept. meningitidis** Bonome, which is closely related to the Strept. pyogenes, to be the cause, still other authors, and especially Jäger, have described as the exciting agent, an organism which is indeed *very closely related to the Strept. lanceolatus*, but which, it is said, can be clearly separated from it. The statements of different authors diverge very widely as regards details.

The cultures are often indistinguishable morphologically from the Strept. lanceolatus, but they remain alive and capable of transplantation for a longer time (seventeen to forty-three days). Some authors found growth to occur upon potato; many obtained even strikingly luxuriant, moist, yellowish-gray cultures upon glycerin-agar, resembling the Micr. tetragenus (68, III and IV) (Mayer, Münch. med. Wochenschr., 1898, 1111), and we received such cultures from Jäger in December, 1896. C. Fränkel cultivated, on the contrary, an exceedingly delicate growth, which only grew with certainty upon agar smeared with blood. Such a culture we obtained from Kràl.

The following points are asserted to be of diagnostic value: The organisms, sometimes as diplococci and tetrads, sometimes as short chains, lie oftentimes in groups *within the pus-cells, especially also within the cell nuclei.* They possess more or less distinct capsules. According to the beautiful investigations of v. Hibler, the most variable pathogenic cocci and bacilli are found in the cells, so that this property is not at all characteristic (C. B. XIX, 33). In smears from the pus and from cultures, they sometimes stain well by Gram's method, but more often poorly, and in sections are not stained (alleged contrast to the Strept. pyogenes and lanceolatus). In the chain form of the organism it is said to be characteristic (Jäger) that the individuals are so arranged that the line separating the diplococci extends in the direction of the chain. But as Stolz has pointed out, exactly similar pictures occur in typical Strept. lanceolatus and Strept. pyogenes (C. B. XXIV, 337). We found such pictures exquisitely shown in a streptococcus growing in a putrid mixture. With this state of affairs it is difficult to consider the Strept. intracellularis a single organism, since the forms which at times resemble the Micr. gonorrhœæ, at times the Strept. lanceolatus and pyogenes, and at times the Micr. tetragenus lack a common characteristic.[1]

The organism is said to be found only in the meningeal pus, nasal mucus, sputum, and urine of men who are affected with epidemic cerebrospinal meningitis. Recently, A. Schiff claims to have isolated it from the nose of patients without meningitis (C. B. XXV, 437). C. Fränkel cultivated it from eyes apparently affected with diphtheria (Z. H. XXXI, 221). Together with the Strept. intracellularis there occur mixed infections by the Strept. pyogenes and Strept. lanceolatus. Certainly at least a considerable portion of the cases of cerebrospinal meningitis are caused by the Strept. lanceolatus alone.

Regarding the *cerebrospinal meningitis of domestic animals*, conflicting statements are also encountered; here also it is possible that different related infectious agents take part in the main epidemics. (Consult Siedamgrotzky and Schlegel, C. B. XX, 694, and Schneidemühl, C. B. XXIII, 892.) It is interesting that Johne found in an epidemic disease of horses an organism which Jäger declared identical with the Strept. intracellularis. The organism was pathogenic for guinea-pigs, horses, and goats. (Consult Councilman, Mallory, and Wright, Amer. Jour. of Med. Sciences, March, 1898.—Ed.)

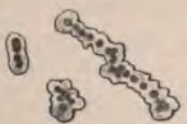

Fig. 13.—Strept. involutus (from a photograph by Kurth); partly schematic.

Streptococcus involutus (Kurth).

Synonym.—Streptococcus of foot-and-mouth disease.

Literature by Kurth (A. G. A., Bd. VIII, 1893, 439–465).

Upon gelatin, etc., indistinguishable from the Strept. pyogenes; on the contrary, bouillon which is rendered diffusely cloudy by some cultures, and only presents a sediment with others, often contains cells of strikingly elongated, vesicular, spindle form. No spontaneous motion.

Two especially striking characteristics are present in serum or serum mixtures:

1. In fluid *serum* or serum bouillon there develops in the upper part of the tube a pale-yellow creamy layer, which upon microscopic examination reminds one at first of anything else rather than micro-organisms, but on further examination the following is learned:

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[1] The following is said to be characteristic: Agar cultures after five to six transfers cease to grow, and the organism generally does not grow on cow's serum.

The luxuriant, waxy, shining masses consist of dense zooglea of streptococci which are surrounded by very extensive, enormously swollen capsules, which do not stain with anilin dyes. The capsule formation occurs best upon calves' serum, but is also seen upon sheep's serum.

2. Upon *plates* prepared with 10 c.c. of agar, and 2 c.c. of serum,[1] both warmed to 40°, there is found about each of the small pure growths a halo of strongly refracting granules, which are doubtless composed of the same material as forms the capsules of the single cells. How these spheres originate and whence they arise, Kurth is unable to say.

Kurth had already stated that the Micr. pyogenes and Micr. tetragenus can furnish similar pictures upon calves' but not upon sheep's serum. He found later that also streptococci not connected with foot-and-mouth disease, although rarely, furnish similar serum cultures. The organism has nothing to do with foot-and-mouth disease (see Appendix).

Streptococcus Mesenterioides (Cienkowski) Migula.

Synonym.—Leuconostoc mesenterioides Cienkowski.
Ordinary Name.—Frog-spawn fungus of sugar factories.

Literature.—Zopf and Liesenberg, "Beiträge zur Physiol. u. Morph. niederer Organismen," Heft. I, Leipzig, 1892; C. B. XII, 659.

The organism grows upon nutrient media free of grape- and cane-sugar, like the Strept. pyogenes[2] microscopically and macroscopically; in a stab of gelatin containing grape- or cane-sugar, on the contrary, it grows upon the surface as a luxuriant deposit consisting of dense, whitish, jelly-like clumps, which possess a "strong glassy luster at the

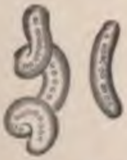

Fig. 14.—Strept. mesenterioides (after Zopf).

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[1] The serum must not be sterilized with chloroform, but only by heat.

[2] Liesenberg and Zopf call these forms **Strept. mesenterioides var. nuda.**

summits," and along the stab as luxuriant stalactite-like masses. The colonies are at first cartilaginous, then moist, and finally pap-like. Upon grape-sugar agar plates the superficial colonies are warty and luxuriant and spread like a wrinkled film ; the deep ones are at first smooth and later sago-like warty balls.

Microscopically the form upon sugar media presents tough, thick, gelatinous capsules (of dextran, compare p. 30).

The gelatinous covering protects for fifteen minutes against 75°. All the varieties of sugar ordinarily employed undergo fermentation, with the formation of gas and acid. The fungus formerly was often the cause of the very troublesome frog-spawn fermentation of sugar solutions in sugar factories.

Leuconostoc lagerheimii Ludwig consists of small (0.6 μ to 0.8 μ) cocci within thick capsules. It causes alcoholic fermentation in the slimy secretion from oaks. The organism is said to occur also without envelopes as short rods with flagella (?).

2. Sarcina (Goodsir).

The cells divide (at least upon suitable nutrient media —hay decoction, bouillon) in regular succession in three directions of space, and remain grouped in larger or smaller cubical families.[1]

The boundary of this genus is not sharp, although the sarcina is held by many authors (Nägeli!) as an especially natural genus. Many varieties only produce true cubical arrangement upon certain nutrient media, and it appears that this property may also be acquired or lost (compare Sarc. rosea). In the case of varieties with incomplete packet formation there is always doubt whether they belong to the sarcina or micrococcus. It is our conviction that the sarcina is connected with the micrococcus by unbroken transition forms, and is only separated arbitrarily. Examples follow.

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[1] We call eight cubically arranged cocci a *packet*; cubical combinations of packets, *bales of packets*; irregular combinations, *heaps of packets*.

The synopsis of definitions and the descriptions may be prefaced as follows: All the sarcinæ which we have investigated grow—to be sure, in part very imperfectly—also anaerobically, and then produce H_2S, in from barely perceptible to large quantities. Aerobically, H_2S is not produced in 2% peptone bouillon by all, and in marked quantity only by those where we expressly state it. A minimal formation of indol occurs with all. In grape-sugar bouillon, with few exceptions, only a little acid is formed in six days (lactic acid), about 0.8 c.c. normal acid to 100 of bouillon. Many convert urea into carbonate of ammonia.

It can not be doubted that sarcinæ can cause cloudiness and souring of beer (Lindner). (Compare Schönfeld, C. B. L. IV, 865.) These are said to originate especially from horse-manure.

All sarcinæ stain well by Gram's method. Beautiful pictures are also obtained by staining with a solution of fuchsin and differentiating with acetic acid. It is important always to observe the fresh preparation in a hanging drop. One must guard against mistaking tetrads (or eight-celled cubes) for single cells, which quite easily occurs, especially with deep staining.

We have not made statements regarding the size of sarcinæ, since we here found especially varying results. It impresses one as if the cells often grew very large and then in rapid succession divided into eight parts.

Endospores we have been unable to find except in Sarc. pulmonum Hauser.

Spontaneous movement has not been observed in any of the sarcinæ examined by us with the exception of the Sarc. pulmonum, but often strikingly marked molecular motion was present, which continued in sublimate solution. The Sarc. mobilis Maurea, obtained from Král, was always non-motile and devoid of flagella.

In many varieties cultivation in fluid nutrient media (hay decoction and bouillon) led to the formation of packets and bunches of packets, which were otherwise formed with difficulty or not at all. When no packets are produced upon these nutrient media, one will seek them in vain upon solid nutrient media. The macroscopic appearance of the bouillon cultures is of little value in differentiating species, as it seems that most varieties finally produce a more or less viscous or friable sediment in the clear bouillon, and that in the same variety the character of this sediment varies. The precipitate either forms upon the bottom or on the walls and bottom, without the bouillon becoming cloudy; or the precipitation is preceded by a longer or shorter diffuse cloudiness of the bouillon. The bouillon takes on in some varieties (Sarc. alba), *but not always*, a characteristic gummy, viscous quality.

The following presentation is dependent not only upon our own studies, but upon the critical elaboration of the material, which Dr. Stubenrath cultivated during about two years under our direction, and upon which he has reported in a monograph, "Das Genus Sarcina," München, 1897. The literature is there extensively presented.

Space does not allow us to enter more into particulars concerning the uncritically described and very numerous varieties of Henrici[1] and Gruber[2]. Stubenrath (*l. c.*) has referred to the fact that those contributions, in a work which does not at all consider the variation of bacteria, have loaded us with many names, but that our knowledge is scarcely advanced thereby.

Key to Recognition of the Sarcinæ.

I. WITHOUT PIGMENT PRODUCTION UPON AGAR AND GELATIN.

(*a*) Potato growth delicate, brownish-yellow from the first. Gelatin and agar growth, delicate, finely notched and wrinkled. Young cultures motile, old cultures often with spores. **Sarc. pulmonum** Virchow, page 155.

(*b*) Potato growth always remains white or grayish-white.

(*α*) Gelatin plate magnified sixty times; very finely granular; limited liquefaction. No formation of large regular bales of packets. **Sarc. alba** Zimmermann, page 160.

(*β*) Gelatin plate magnified sixty times; medium-sized granules; liquefaction more rapid; formation of beautiful regular bales of packets. **Sarc. canescens** Stubenrath, page 159.

II. UPON AGAR AND GELATIN GRAYISH-YELLOW, GREENISH-YELLOW TO CHROME-YELLOW.

(*a*) Gelatin plate magnified sixty times; very finely granular;

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[1] Henrici, "Beitrag zur Bakterienflora des Käses" (A. K. Bd. I, 1).

[2] Gruber, "Die Arten der Gattung Sarcina" (A. K. Bd I, 241).

potato growth chrome-yellow, glistening; no large regular bales of packets formed. **Sarc. flava** de Bary, emend. Lehmann and Stubenrath, page 159.

(*b*) Gelatin plate magnified sixty times; medium-sized granules; beautiful regular bales of packets formed. This group contains transitions from flava to lutea, and from the yellow to the white forms.

(*α*) Potato growth; at first dark gray, only later yellowish-brown. **Sarc. livido-lutescens** Stubenrath, page 159.

(*β*) Potato growth; from the beginning grayish-yellow, at other times very similar. **Sarc. equi** Stubenrath, page 158.

(*γ*) Like Sarc. equi, but motile from long flagella, sometimes somewhat fluorescent. **Sarc. mobilis** Maurea, page 160.

(*c*) Gelatin plates magnified sixty times are coarsely granular. Formation of beautiful regular bales of packets; potato growth, from beginning, luxuriant lemon-yellow. **Sarc. lutea** Flügge, emend. Lehmann and Stubenrath, page 157.

III. UPON AGAR AND GELATIN ORANGE-YELLOW. **Sarc. aurantiaca** Flügge, page 160.

IV. UPON AGAR AND GELATIN BROWNISH TO BROWNISH-YELLOW.

(*a*) Agar streak succulent, broad, reddish-brown. **Sarc. cervina** Stubenrath, page 162.

(*b*) Agar streak thin, finely notched, and furrowed, yellowish-brown, transparent. **Sarc. fulva** Stubenrath, page 156.

V. UPON AGAR AND GELATIN BRIGHT ROSE-RED.

(*a*) Gelatin and agar streak rose-colored; sarcina form observed only upon hay decoction. **Sarc. rosea** Schröter, emend. Zimm., page 162.

(*b*) Gelatin and agar bright red; sarcina form observed by us only once upon hay decoction. **Sarc. erythromyxa** Král, page 162.

That it will *always* be possible to distinguish the "forms" presented in the key, we can not certainly claim, since in spite of the observation during two years of very numerous forms, we have reached no final judgment concerning the extent of variability and perhaps the occurrence of transition forms.

Leaving the chromogenesis out of account, we can cite at least two striking examples of their variability (compare Sarc. variabilis and mobilis); thus, the following appears the natural relationship:

1. Sarcina flava,—therefrom is the white form, Sarc. alba.

2. Sarcina equi,—therefrom is the white form, Sarc. canescens.

Between equi and canescens Sarc. livido-lutescens and Sarc. variabilis reestablish a connection.

The varieties Sarc. flava, equi, and lutea form a series in which the coarseness of the granules of the culture and the size of the bales of packets continually increase; entirely parallel with this is the series Sarc. alba, variabilis, and canescens.[1]

Sarcina pulmonum (Virchow, Hauser).

(Plate 6, VI–X.)

Literature.—Hauser, "Deut. Arch. f. klin. Med.," XLII, 127; Stubenrath, monograph.

Microscopic Appearance.—Upon the various nutrient media only small and not especially regular bales of packets were formed.

Motility.—Young cultures exhibit exquisite waltzing movement (Hauser) dependent, according to Job (Diss. Würzburg, 1896), upon not very numerous, long, coiled flagella. Older cultures, and quite often also young ones, exhibit no motility.

Growth.—Very slow even at incubator temperature.

Gelatin Plates.—(*a*) *Natural size:* Extremely small, roundish, yellowish-grayish-white, punctiform colonies.

(*b*) *Magnified fifty times. Superficial:* At first roundish, smooth border; gray, almost opaque, not different from the deep ones. After two to three weeks the peripheral part is lost from sinking in of the colony, and it then appears torn, and (especially at the edge) transparent, coarsely crummy. Packets are not to be made out; color gray. *Deep:* Roundish, gray, opaque, without any visible internal structure (6, VIII).

Gelatin Stab.—At first thread-like, and only after a long time crummy; gray to yellowish-gray. *Surface growth:* After twenty days, 2 mm. to 3 mm. wide, gray, transparent, roundish, serrated, faintly shining. Later it begins to sink in (6, VI).

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[1] We have not described a **Sarc. ventriculi Goodsir** because the description given by Falkenhain (Arch. für exp. Path. u. Phar. XIX, 339), which was copied by Gruber, does not agree accurately with any of our forms, and, as Oppler (Münch. med. Wochenschr., 1894, No. 29, 570) first pointed out, the stomach contains a whole series of sarcinæ. (For details thereon, see Stubenrath.)

Agar Plates.—(*a*) *Natural size:* Like gelatin plate, only somewhat whiter.

(*b*) *Magnified fifty times. Superficial:* Round, light to dark gray, periphery lighter, transparent; tetrads visible as tiny crumbs. *Deep:* Roundish, dark, finely granular.

Agar Stab.—*Stab:* Thread-like, later granular. *Surface growth:* Grayish-white, shining, slightly elevated; after three weeks 4 mm. to 5 mm. in diameter.

Agar Streak.—Restricted to the streak; rather scanty growth; grayish-white, transparent, wavy, usually made up of single crumbs. Water of condensation clear with slight sediment (6, VII and 5, II).

Bouillon Culture.—Clear, little deposit, friable.

Milk Culture.—Milk very slowly becomes clear, without preceding coagulation.

Potato Culture.—Very poor growth; after three to four weeks a growth 3 to 4 mm. wide, yellowish-gray to brownish, shining, not sharply outlined from the potato (6, IX).

Spores.—Typical, round spores first observed by Hauser; according to Hauser, they stain well.

Occurrence.—So far found only in the air passages of men—for example, in cases of phthisis—apparently as harmless settlers; according to Hauser, are not pathogenic for animals.

The following appears **very similarly** (but always lacks spores and flagella):

Sarcina fulva (Stubenrath).

In microscopic findings upon all nutrient media, in distribution and consistency, liquefaction, etc., almost exactly like the preceding, but is *brownish-yellow to reddish-brown*, and transparent upon agar and gelatin; on the contrary, upon potato scarcely to be distinguished from Sarc. pulmonum. Bouillon becomes turbid, with tough crumbly sediment. Grown with oxygen it forms some H_2S, and rather abundant acid upon grape-sugar bouillon and milk. Upon all nutrient media it forms bunches and bales of packets, but of various sizes.

Cultivated in Würzburg many times from stomach contents and once from preputial smegma; a very striking and slowly growing variety.

Sarcina lutea.[1] (Flügge, emend. Lehmann and Stubenrath).

Microscopic Appearance.—Upon nutrient media typical bales of packets.

Gelatin Plate.—(*a*) *Natural size.* Roundish, punctiform colonies, sulphur-yellow; after ten to twenty days, sinking in (3, v).

(*b*) *Magnified fifty times. Superficial:* Roundish, even-bordered or almost smooth-edged colonies; pale yellow with at first a finely granular and later (eight to ten days) a more coarsely granular structure. After a very long time the peripheral parts separate somewhat and, with higher magnification, individual tetrads are seen (3, VI). *Deep lying:* Roundish, dark yellow, even-bordered, finely granular.

Gelatin Stab.—*Stab:* Thread-like, with relatively few coarse granules. *Surface growth:* Irregularly circular, with a moist luster, somewhat elevated, sulphur, lemon-, or even deep yellow. After ten to twelve days the superficial growth sinks down. Liquefaction at first extends in a funnel form and later as a cylinder; however, we have cultivated almost non-liquefying forms (3, I).

Agar Plates.—(*a*) *Natural size. Superficial:* Round or roundish, even-bordered, somewhat elevated; sulphur-yellow, with a moist luster. *Deep:* Roundish to whetstone-shaped (3, VII).

(*b*) *Magnified fifty times. Superficial:* Roundish almost even-bordered colonies; periphery delicately punctate; peripheral zone transparent, pale yellowish, becoming darker toward the center; finely to coarsely granular (3, VIII). *Deep:* Like those upon gelatin with coarser granulation.

Agar Stab.—*Stab:* Thread-like, finely to coarsely granular, at times after a long while ray-like outgrowths; yellow. *Surface growth:* Roundish, wavy, even border, somewhat elevated, moist, of consistency of butter; sulphur- to chrome-yellow (3, III).

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[1] **Plate 6, Figs. I to V, illustrating the Micr. luteus Cohn, serve exactly as well for the Sarc. lutea, except figure III, where the bales of packets are absent. Also Plate 3 would pass for the gelatin plate cultures, except for the finely granular structure (3, VIII); a somewhat lighter form (5, IV).**

Agar Streak.—Similar; water of condensation clear; whitish-yellow precipitate (3, II).

Bouillon Culture.—Clear; abundant sediment.

Milk.—Coagulated after forty-eight hours.

Potato Culture.—Wavy surface growth, often much elevated, shining, especially in old cultures having larger or smaller elevations; in young cultures with a moist luster, later dull, sulphur-, chrome- and more rarely grayish-yellow, limited to the line of inoculation, only extending a little more widely after a long time (3, IX).

Chemical Activities.—In peptone-bouillon there is formed some H_2S and a trace of indol. The yellow pigment is a lipochrome. In grape-sugar bouillon some acid is formed.

Distribution.—Very common variety in the surroundings of men, especially in the air. In Würzburg every plate from air contained it.

Remarks.—

The numerous forms isolated by Dr. Stubenrath which belong here, we group under the following varieties:

(α) **Typica** (Lehmann and Stubenrath). The colony on gelatin may be recognized upon the plate by a marked cleaving of the border, and even with progressing liquefaction of the gelatin the round form is not essentially changed.

(β) **Compacta** (Lehmann and Stubenrath). The colonies on the gelatin plate are very luxuriant, roundish, and so compact that a border-zone can not be distinctly seen. As this form also causes almost no liquefaction of gelatin, the colonies lie upon the plate as a tough film in the scarcely depressed gelatin.

(γ) **Diffluens** (Lehmann and Stubenrath). This form shows upon all nutrient media a very marked tendency to spread out. Upon gelatin plates, which are liquefied quite rapidly, the colony spreads as a very much fissured, readily disintegrating mass.

Sarcina equi (Stubenrath).

In all respects similar to the Sarc. lutea, but is differentiated:

1. By medium-sized granules, not coarse granules, in the gelatin plate.
2. Less perfectly formed bales of packets.
3. More grayish-yellow color on all nutrient media; little lique-

peatedly by Dr. Stubenrath in the urine of various horses in Würzburg. In cultures it remained constant for a year, the original active liquefaction only being somewhat lessened. The three following are subspecies or varieties:

Sarcina livido-lutescens (Stubenrath).

Like Sarc. equi, but young potato cultures for ten days and more are gray to reddish-gray; after twenty days they become brownish-yellow in the center, and after a month throughout the entire culture. The constancy of this characteristic was observed for a year. In a case of enteritis it was grown abundantly from the stool by Dr. Stubenrath.

Sarcina canescens (Stubenrath).

Differentiated from Sarc. equi only by constant gray color and somewhat coarser granulation (larger bales of packets) upon all nutrient media (5, VIII).

Sarcina variabilis (Stubenrath).

This form, isolated from gastric contents, appears to us to be very interesting. It is differentiated from the Sarc. equi only by more marked liquefaction of gelatin and by the property of furnishing on the various nutrient media *sometimes yellowish-gray, sometimes pure gray colonies.* Upon plates one often obtains gray and yellowish colonies side by side, but this is alike repeated whether one inoculates from gray or yellowish colonies.

Sarcina flava (de Bary, emend. Lehmann and Stubenrath).

(Plate 3.)

Upon all nutrient media it is habitually very similar to the Sarc. lutea, being yellow to greenish-yellow. The principal difference lies in the very finely granular gelatin plate colonies when magnified sixty times. When magnified one thousand times, this fine granulation is seen to depend upon very small bales and heaps of packets.[1] We have observed one form that is more luxuriant and distinctly liquefying, and one that is more delicate, leaving the gelatin still solid after weeks, growing feebly upon all nutrient media. It has been repeatedly cultivated from gastric contents.

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[1] The **Sarc. flava**, obtained from Král, Dr. Stubenrath found to form upon all fluid and solid nutrient media, usually only bunches of cocci, rarely tetrads, and never true bales of packets.

Sarcina alba (Zimmermann).

If one imagines the very feebly liquefying forms of Sarc. flava without formation of pigment, then one obtains the Sarc. alba likewise with variable liquefaction. The growths on the various nutrient media are white to grayish-white, usually very delicate. Microscopically this variety is not distinguishable from Sarc. flava, so that, when transition forms are found, they appear only as varieties.

Sarcina mobilis (Maurea).

The inoculation from an original culture sent to our institute by Král resembled our Sarc. equi, very markedly in the color (grayish-yellow), upon all the nutrient media and in its slow but always distinct liquefaction, yet the granulation in the gelatin plate cultures, magnified sixty times, is still finer, somewhat like the Sarc. flava, midway between this and the Sarc. equi.

Now and then a yellowish-green fluorescence occurs upon agar and gelatin, which we have observed in no other sarcina. Although the granulation is fine, beautiful packets occur upon all nutrient media. We were not able to see the spontaneous motion described by Maurea, nor could we stain flagella. *Our variety appeared to have lost the ability to produce flagella.* R. O. Neumann has grown a white and a yellow culture. Migula, who has seen the flagella, produced a picture of them. Sames has described and illustrated by photographs a gray variety of sarcina, which is actively motile and provided with numerous long flagella, obtained from dung-water (C. B. L. IV, 664). It may be called Sarc. fimentaria L. and N.

Sarcina aurantiaca (Flügge, Lindner).

(Plate 4.)

Microscopic Appearance.—Beautiful bales and bunches of packets upon all ordinary nutrient media.

Gelatin Plate.—(*a*) *Natural size:* Orange-yellow, small, round, dot-like colonies, which soon sink into the gelatin. After five to six days the peripheral part breaks up and portions of the colony swim about in the plate-shaped rea of liquefaction. Thus the colony appears whitish range (4, v).

(*b*) *Magnified fifty times. Superficial:* At first round, most even-bordered colonies, pale to deep yellow, strucreless or finely granular. The shallow funnel-shaped pression appears gray. Later the border of the colony broken, fringed, and wavy, and when magnified a hunred times presents tetrads that are single or joined in clumps. At this stage the peripheral zone is perfectly transparent (4, VI). *Deep:* Like young superficial ones.

Gelatin Puncture.—The colony sinks in after thirty-six hours, so that usually the gelatin presents the appearance of a contracting blister. The stab-canal presents a funnel-shaped liquefaction, the wall being beset with fine fragments of the colony. At the bottom of the funnel is an orange sediment (4, I).

Agar Plate.—(*a*) *Natural size. Superficial:* Round or roundish colonies, even-bordered, somewhat elevated, orange with a moist luster. *Deep:* Roundish to whetstone-shaped, similarly colored (4, VII).

(*b*) *Magnified fifty times:* Irregularly round; central zone opaque, brownish-green, toward the border lighter and more yellow, coarsely granular; with stronger magnification individual tetrads are to be seen (4, VIII).

Agar Stab.—*Stab:* Thread-like, coarsely granular. *Surface growth:* Irregularly round, wavy, somewhat elevated, orange-yellow to orange-red, with a consistency like butter, shining moistly (4, IV).

Agar Streak.—Like agar stab; water of condensation clear; yellowish sediment (4, II).

Bouillon Culture.—Unevenly turbid, many single flocculi, abundant sediment.

Milk Culture.—Milk is coagulated, and later the coagulum is again liquefied.

Potato Culture.—Luxuriant growth, with rough, wavy border; after a longer time distinctly elevated; reddish-orange, especially in old cultures, and then is usually dull and irregular like a strawberry. In earlier stages it is yellowish-orange and at times shining. Very similar to the Micr. pyogenes aureus (4, IX). (Compare also 8, IX.)

Chemical Activities.—The orange-yellow pigment is a lipochrome. In grape-sugar bouillon there is feeble acid production. When grown aerobically upon nutrient media without sugar, there is produced no H_2S, but a trace of indol.

Occurrence.—*Outside the organism:* Very common in the air; almost upon every plate made from the air in Würzburg.

Related Varieties.—All orange-yellow sarcinæ, which were cultivated in our institute could be easily designated as Sarc. aurantiaca; moreover, we can not differentiate **Sarc. aurea** Macé, **Sarc. aurescens fusca** and **fuscescens** Gruber from Gruber's description.

Sarcina cervina (Stubenrath).

(Plate 5, I.)

Gelatin plate colonies, macroscopically, at first are whitish, after four to five days pale brown, somewhat moist, slowly becoming surrounded by a zone of liquefaction. Magnified sixty times: with coarsely granular projections, gradually breaking up at the edge into coarsely granular, cloudy masses. Gelatin stab—superficial growth small, pale brown, very slowly sinking in. Stab—faint, thread-like, finely granular. Agar plate—similar to that on gelatin. Agar streak—broad, moist, elevated, yellowish-brown (5, I). Potato culture—brownish-white. Magnified one thousand times, it is seen to consist of mostly irregular bales of packets, which appear a light brownish color. This variety was once isolated from the gastric contents in a case of carcinoma.

Sarcina erythromyxa (Král).

(Plate 5, III.)

Literature.—Král (list of the bacteria handed over); Micr. erythromyxa Overbeck (Nov. Act. der Leop.-Carol, Bd. 55, No. 7, 1891). Good description by Zimmermann (II, 70).

Magnified one thousand times, usually only cocci, diplococci, and tetrads; only once did we obtain upon hay decoction a beautiful formation of regular bales of packets.

Upon gelatin plates (natural size) the colonies are at first a lively greenish color, then beautiful carmine- to vermilion-red, and moist. Magnified sixty times, almost without granulation; at the edge the red colonies are usually transparent and finely notched. There is no liquefaction. Gelatin stab, agar stab and streak, and potato cultures gradually develop as an intensely red, shining, rather spare growth. Upon milk a red growth forms on the surface, and the milk slowly becomes clear without preceding coagulation. Bouillon becomes cloudy with a coarse, crumbly sediment and at times a pellicle. Moderate production of acid on grape-sugar bouillon.

Sarcina rosea. J. Schröter emend. Menge (B. vi, 596) and Zimmermann (ii, 58).

The description of this organism (5, VI) coincides *absolutely* as regards its growth upon all nutrient media with that given for the *Micr. roseus* (p. 190); the illustrations in Plate 11 also are as good for the

Sarc. rosea. On the contrary, the culture we obtained from Král, upon agar, hay decoction, and urine, contained bales of packets.

3. Micrococcus (Cohn).

The cells divide irregularly in various directions and lie singly, in pairs, in fours, or, finally and indeed mostly, in irregularly bunched heaps. In this class are included all cocci which are not undoubted streptococci or sarcinæ.

Key to the Determination of the Micrococci.

I. Does not grow upon any of the *ordinary* nutrient media aerobically or anaerobically; on the contrary, grows upon human *blood-serum*, agar smeared with *blood*, etc. Microscopically, pairs of kidney-shaped cocci, connected by a usually *broad*, unstained cement line; round forms are more rare. *Does not stain by Gram's method.* Never found except in human body or its secretions. **Micr. gonorrhœæ** Neisser, page 164.

II. Poor growth upon the ordinary nutrient media and upon serum. Besides cocci, often rod-forms (!) are found, which may be four times as long as wide; not stained by Gram's method. **Micr. melitensis** Bruce, page 168.

III. Upon the ordinary nutrient media a thick, white, abundant growth, sometimes forming tetrads. In animal body always tetrads with marked gelatinous capsules. **Micr. tetragenus** Gaffky, page 171.

IV. Grow upon ordinary nutrient media; always spherical; no tetrads in animal body.

A. *Upon gelatin and agar, do not produce pigments (white to gray varieties).*

(*a*) Gelatin not liquefied; colonies in plate roundish with no outgrowths.

- (*α*) Growth on gelatin and agar thick, pure white; not pathogenic; arrangement irregular.
  1. Individuals rather large. **Micr. candicans** Flügge, page 169.
  2. Individuals very small. **Micr. aquatilis** Mead Bolton, page 171.
- (*β*) Similar to *α*, but color yellowish-gray, not pure white. **Micr. rosettaceus** Zimmermann.
- (*γ*) Growth upon gelatin thin and iridescent. **Micr. concentricus** Zimmermann, page 174.

(*b*) Gelatin not liquefied; delicate white tendrils extend outward from the deep colonies in gelatin plates and from the gelatin stab. **Micr. viticulosus** Katz, page 174.

(*c*) Gelatin liquefied; plate and stab cultures without tendrils or branches. **Micr. pyogenes** *γ* **albus** (Rosenbach), L. and N.,[1] page 187.

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[1] Compare Micr. Freudenreichii Guillebeau, Micr. acidi lactis Krüger.

(*d*) Gelatin liquefied ; colonies in plates with teeth or branches.

(*α*) Gelatin stab without branches. The funnel of liquefaction in the gelatin plate cultures is surrounded after a few days with a yellowish-white circle of ragged points and teeth. (Compare also Micr. corallioides Zimmermann.) **Micr. coronatus** Flügge, page 175.

(*β*) In gelatin stab are branches. The colonies in the gelatin plate present a circle of pretty rays. **Micr. radiatus** Flügge, page 176.

B. *Upon gelatin and agar, sulphur-yellow to lemon-yellow pigment is produced.*[1]

1. Colonies in gelatin coarsely granular ; liquefaction rapid. **Micr. luteus** Cohn, emend. L. and N., page 176.

2. Colonies in gelatin finely granular ; liquefaction rapid. **Micr. flavus** Flügge, L. and N., page 178.

3. Colonies in gelatin finely granular ; no liquefaction. **Micr. sulfureus** Zimmermann, page 178.

C. *Upon gelatin and agar, formation of brownish-yellow pigment.* **Micr. badius**, L. and N., page 178.

D. *Upon gelatin and agar, orange-yellow to grayish-orange.*

(*a*) Agar streak uniformly orange-yellow.

(*α*) Gelatin liquefied, pathogenic. **Micr. pyogenes *α* aureus** (Rosenbach) L. and N., page 181.

(*β*) Gelatin not liquefied ; found in air. **Micr. aurantiacus** Cohn, page 189.

(*b*) Agar streak, mottled gray and orange. **Micr. bicolor** Zimmermann, page 189.

E. *Upon gelatin and agar, rose to crimson.*

(*a*) Rose to cherry-red ; upon potato, slight growth. **Micr. roseus** (Bumm), L. and N., page 190.

(*b*) Rose to cherry-red ; upon potato, broad, dry growth. **Micr. cerasinus** (List) L. and N., page 193.

(*c*) Scarlet red. **Micr. erythromyxa** Zopf, page 193.

F. Upon gelatin and agar, cobalt blue. **Micr. cyaneus** (Schröter) Cohn, page 193.

Micrococcus gonorrhœæ (Neisser) (Flügge).

(Plate 10.)

Synonyms.—Gonococcus (Neisser), Diplococcus gonorrhœæ Bumm, Micrococcus Gonorrhœæ Schröter.

Most Important Literature.—A. Neisser, "C. f. med. Wiss.," 1879, 497; Bumm, "Der Mikroorganismus der gonorrh. Schleimhauterkrankung," Wiesbaden, monograph, 1885; Wertheim, "Archiv für Gynäkologie," XLII, 1892, 1; Wassermann, XXVII, 298. Latest exhaustive review of the literature by Fouleston, "Transact. of the Inst. of Prev. Med.," Vol. I, 1898.

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[1] Compare also the pathogenic Micr. ascoformans Johne, the Micr. pyogenes β citreus (Passet), and the Micr. ochroleucus Prowe, which is said to form spores.

Microscopic Appearance.—They usually occur as pairs of organisms, somewhat kidney-shaped, united by a lenticular cement material which is often quite broad. A pair is 0.8 μ to 1.6 μ long, and 0.6 μ to 0.8 μ broad (10, x).

Staining Properties.—By the usual staining methods, best with Löffler's methylene-blue. It is not stained by Gram's method, which is very important, as it *differs in this from almost all cocci.* Recently many authors have claimed that gonococci at times stain by Gram's method. Weinrich (C. B. XXIV, 258), who discusses the entire literature, maintains that prompt decolorization is always obtained if the preparations which are stained with anilin- or carbol-gentian violet solution are brought directly into Lugol's solution without washing with water and then into truly absolute alcohol. If one desires a contrast color in the cells, a weak aqueous solution of Bismarck brown is employed after the cover-glass has been brought from the absolute alcohol to water.

Relation to Oxygen.—Facultative anaerobe.

Requirements as Regards Temperature and Nutrient Media.—Grows only at incubator temperature, best at 36°. The extremes are from 25° to 39°. Growth on all nutrient media very slight, and frequent transfer is necessary to keep it alive. It is one of the most difficult varieties to keep permanently in culture. It is remarkable that cultures die at room temperature in forty-eight hours.

The growth of gonococci upon the ordinary nutrient media is not to be undertaken.[1] Smears are to be made upon one of the following nutrient media (3, 4, and 5 may also be used for plates):

1. Ordinary nutrient agar, smeared over with **human blood** (from the sterilized finger-tip of the investigator, Abel). To be recommended as the simplest method.
2. **Human blood-serum** (from placenta or obtained by venesec-

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[1] The statements of Turró regarding the cultivation of the gonococcus upon acid gelatin, the successful inoculation in the dog, and the liquefaction of alkaline gelatin could be verified by no one.

tion). The serum of animals is usually unsuitable, and upon it the growth is always very slight (Bumm).

3. We have (with Kiefer and Menge) obtained very good results with a nutrient medium, always prepared when used by mixing 2% agar (with 1% peptone and 5% glycerin), which has been liquefied and cooled to 50°, with half its volume of **ascites fluid** or the fluid from ovarian cysts (see Technical Appendix).

4. We have had no good results with a nutrient medium consisting of urine and glycerin-agar, or with simple glycerin-agar.

5. Wassermann recommends the following as the best gonococcus nutrient medium: 15 c.c. of swine serum, as free as possible from hemoglobin, is placed in a small Erlenmeyer flask, diluted with 30 to 35 c.c. of water, and to this is added 2 to 3 c.c. of glycerin and, finally, 0.8 to 0.9 gm. (about 2%) of nutrose (casein-sodium phosphate). The whole is now mixed as thoroughly as possible by shaking and heated, with constant stirring, over a free flame to the boiling-point. The previously turbid fluid becomes clear upon boiling, and may be properly heated in the moist oven to render it sterile. The addition of the nutrose prevents the precipitate from the serum. In the preparation of cultures an equal quantity of 2% agar cooled to 50° is poured into the flask, the two mixed and poured into a Petri dish. As soon as it becomes solid, the nutrient medium is ready for use. The cultures are the more luxuriant, the fresher the case of gonorrhea and the less it has been treated. Growth is favored by the admission of air.

Plate Cultures.—(*a*) *Natural size:* Like the streak culture.

(*b*) *Magnified fifty times:* The great delicacy of the colonies is characteristic of the gonococcus. Upon blood-agar and serum-agar, as well as upon ascites glycerin-agar, the colonies are transparent gray with a shade of yellow, exceedingly delicate, scarcely at all, or only very finely, granular. Often at the periphery the colony is indistinguishable from the medium. They are very slightly elevated (10, III, below; V, II). At this stage they are very similar to the Strept. lanceolatus. In older colonies the border, which was formerly smooth, becomes partly wavy and irregular, the structure somewhat granular (10, II), and eventually even moruloid (10, IV), yet it is always more delicate than the streptococcus. If an inoculation is made upon agar smeared with blood the colonies appear principally at the periphery of the streak, like a cloud, or, upon becoming larger, press the blood aside (10, II). The same happens if gonorrheal or blennorrheal pus is placed upon ascites-glycerin-agar. The pus pushed aside forms septa, between which the colonies develop. This is a very characteristic picture (10, VI).

Streak Culture.—Transparent gray deposit, perhaps with a trace of dirty yellow, somewhat elevated especially at the edge. It has an oily but not a moist gloss. It gives the impression as of mucus upon the surface, thus differing from the streak cultures of other delicately growing organisms, as the Strept. pyogenes or lanceolatus.

Toxins.—Upon nutrose-serum bouillon Wassermann obtained vigorous cultures, which were still poisonous after being killed. The gonotoxin from the bodies of gonococci is very resistant to heat and alcohol, kills mice, produces a doughy infiltration in rabbits and mice, which often ends in necrosis. With large doses systemic effects occur (compare Nicolaysen, C. B. XXII, 305). Gonotoxin injected subcutaneously was without effect in chronic gonorrhea in man. The marked reaction following the injection did not become less upon repeating the injection.

The gonotoxin explains the gonorrheal secretion. Also some points in the history of chronic gonorrhea may be explained by the fact that for a long time a few gonococci slowly multiply and die and keep up a suppuration almost free of gonococci, but that they may increase more actively after any injury, irritation, etc., of the tissue and an acute exacerbation of the process, with abundant formation of toxin and large numbers of gonococci, may develop.

Also, the filtrate from cultures of gonococci in ascites bouillon was irritating, according to Schäffer, producing a suppuration upon the urethral mucous membrane (C. B. XXIII, 708).

Distribution.—(*a*) *Outside of the organism:* Never, except upon linen, towels, etc., soiled by those with the disease.

(*b*) *In healthy organism:* Never.

(*c*) *In diseased organism:* In gonorrhea in the urethra and prostate of men; in the urethra, Bartholinian glands, cervix uteri in women. Cause of vaginitis and urethritis in young girls.

Besides these, in isolated cases it causes endometritis, metritis, salpingitis, oophoritis, peritonitis, proctitis, vesical catarrh, and probably also epididymitis. Cause of blennorrhea neonatorum, rarely of diphtheritic conjunctivitis in children (C. Fränkel); the gonococcus also causes, in adults, severe conjunctivitis, rarely rhinitis and otitis. The gonococcus is often recognized as the cause of arthritis, and more rarely of pleuritis and malignant endocarditis, abscesses, parotitis, periostitis, and bursitis. Now and then these demonstrations are not entirely free from objection.

As influencing the local infection, squamous epithelium is a better protection than cylindric epithelium. The parasite gradually passes through the epithelium into the connective tissue and causes an irritation and inflammation there. No immunity follows recovery from the infection.

In migrating to distant regions of the body, the gonococcus especially follows the lymph spaces and causes inflammations, which lead to fibrous proliferation (for example, urethral stricture).

Experimental Pathologic Experiences.—*In animals:* The results of inoculation are always negative. Large quantities of culture cause toxin inflammations without increase of the cocci, just as is done by the toxins alone.

In man: The production of gonorrhea and conjunctivitis with pure cultures is easily accomplished.

Special Methods of Recognition.—The following peculiarities are to be demonstrated: Diplococci, lying in clumps in the leuokcytes about the nuclei, staining with methylene-blue, and not by Gram's method. Delicate colonies in smears upon blood-agar and serum-agar. The most positive control inoculation is upon the human urethra.

Varieties related to the Micr. gonorrhœæ.

Several varieties have been partially studied by v. Bumm, which may be mistaken for the Micr. gonorrhœæ because of their microscopic form. We will only mention them, as we have not studied them, and will refer to the work of Bumm, already spoken of, for further details.

Micrococcus albicans amplus.—Grows grayish-white upon gelatin, and is larger than the Mic. gonorrhœæ.

Diplococcus albicans tardissimus.—Microscopically is identified morphologically with the Micr. gonorrhœæ, but grows upon gelatin, although very slowly.

Micrococcus subflavus.—See under Micr. pyogenes.

Micrococcus melitensis (Bruce).

Literature.—Durham, Jour. of Pathology, Vol. v, 1898, 377.

Common Name.—Coccus of Malta fever.

A small coccus; in fluids, especially in the incubator, it not rarely forms chains. Cultures at room temperature consist mostly of bacilli,[1] which are from two to four times as long as they are broad. At body temperature cultures of cocci again develop. Non-motile. Do not stain by Gram's method.

At 37° colonies grow slowly upon all nutrient media, being white, hemispherical; upon gelatin at room temperature there is scarcely any growth.

Bouillon at first becomes cloudy, then presents a flocculent precipitate. Milk is not coagulated. Neither gas nor acid is formed from sugar. There is usually an invisible growth upon potato.

In man it causes Malta fever, also in monkeys after cerebral injection. Rabbits and guinea-pigs may be infected, guinea-pigs also intraperitoneally. The serum causes agglutination of the cocci. The elimination of the coccus with the urine, which may continue for months, is interesting.

Micrococcus candicans (Flügge).

(Plate 9, IV-VIII.)

Microscopic Appearance.—Round cocci, lying singly or in bunches, 1.2 μ in diameter. Usually they present a dividing line in the center (9, VIII).

Relation to Oxygen.—Grow well aerobically, and only slightly in the lower parts of shake cultures.

Requirements as to Temperature and Nutrient Media.—Grow at room and incubator temperatures and upon all the usual nutrient media.

Gelatin Plates.—(*a*) *Natural size:* Round or roundish colonies, after eight days at usual temperature being from 2 mm. to 3 mm. in diameter, moistly shining, porcelain-white, slightly elevated. Upon old plates there are always found, besides flatly spreading colonies, those like grains of sand or even conical elevations (9, V).

(*b*) *Magnified fifty times. Superficial:* Round to roundish colonies, even-bordered, extremely delicately punctate, at the periphery partially transparent, becoming opaque, and yellowish-gray to black toward the center. *Deep:* Roundish to whetstone-shaped, opaque, even-bordered, dark (9, VI).

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[1] Thus this organism lies between the families of the coccaceæ and bacteriaceæ.

Gelatin Stab.—Thread-like, granular, white. *Surface growth:* Wavy smooth border, somewhat elevated, shining like porcelain, later somewhat dull, white, with consistency of butter (9, IV).

Agar Plates.—The colonies, when of the natural size or magnified sixty times, are like those in gelatin plates, except that they are somewhat more elevated and more opaque.

Agar Streak.—Slightly spreading, white, oily-looking growth, with a wavy, smooth border, and moderately elevated. Water of condensation clear. White precipitate (9, I).

Bouillon Culture.—Extremely cloudy with moderate sediment; with some forms the bouillon remains clear, and there is formed a pellicle and sediment of greater coherence.

Milk Culture.—Not coagulated in fourteen days, but it becomes very feebly acid.

Potato Culture.—Thick, white, porcelain-like growth, with an oily luster, much elevated, with a wavy border. In time the neighborhood of the growth is discolored gray. The growth of the same cultures upon old potatoes (March) is much drier and more crumbly (9, VII).

Chemical Activities.—Does not liquefy gelatin, forms no gas upon nutrient media containing sugar, and no indol nor H_2S.

Distribution.—(*a*) *Outside the body:* Very common in air, water, milk; everywhere in Germany where it has been looked for.

(*b*) *In organism:* Only epiphytic, for example, in preputial smegma and human hairs.

Forms: We have isolated a Micr. candicans, which differs only from the stock variety in liquefying gelatin feebly.

Related Varieties.—The Staphylococcus cereus albus Passet only differs from this variety in the smaller size of the individuals (perhaps only a forma depauperata from long culture)(from 0.5 μ to 0.8 μ); otherwise it corresponds in all particulars. According to Leube's description (Virch. Arch. 100, p. 560), the **Micr. ureæ** is entirely identical morphologically with the Micr. candicans (0.8 μ); the colonies in gelatin plates at times present sectorial cracks, old cultures have an insipid, pasty smell. Any statement regarding the growth on potato is lacking.

Microccocus aquatilis (Mead Bolton).

We are not familiar with this organism. It is common in the water in Göttingen (Z. H. I, 94), and is characterized by "very small" individuals. The colonies in gelatin plates present something of radial streaks and circular lines, so that rhomboid spaces occur. Further characteristics are not given by Bolton. The organism is able to grow in distilled water. According to Schröter's insufficient description, it may perhaps be identical with the **Micr. candidus** Cohn.

Also the **porcelain coccus** of Escherich from the intestine ("Darmbakterien," p. 90) appears similar; it measures only 0.3 μ.

Micrococcus tetragenus (Koch and Gaffky).

(Plate 7.)

Synonyms.—Micr. tetragenus septicus Boutron, Micr. tetragenus albus Boutron.

Principal Literature.—Koch and Gaffky, "Mitteil. a. d. Gesundh.," Bd. II, 42; Langenbeck's "Archiv," Bd. 28, 500; Boutron's "Thèse de Paris" contains a monograph upon the organism, Reference in C. B. XVI, 971; Teissier, "Arch. de med. exp.," VIII, 14.

Microscopic Appearance.—Roundish or somewhat oval cocci, usually lying in pairs or fours.[1] The size is somewhat variable. Not infrequently one sees but little characteristic cell arrangement in a microscopic preparation, made from a culture. In the animal and human body the arrangement in tetrads is regular, and a rather thick unstained gelatinous capsule surrounds the tetrad. In sections stained by Gram's method the capsule may be counterstained with eosin.

Relation to Oxygen.—Grows well with oxygen, and not so well without.

Requirements as to Temperature and Nutrient

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[1] We have found, on one occasion, in old cultures in hay decoction typical sarcina forms. Unfortunately the observation was not followed further. Contamination is not excluded.

Media.—Grows best at 37°, but also at room temperature, upon all ordinary nutrient media.

Gelatin Plate.—(*a*) *Natural size. Superficial:* Small, irregularly shaped colonies, with even border, whitish, slightly elevated, shining, moist. *Deep:* Uncharacteristic (7, VII).

(*b*) *Magnified fifty times. Superficial:* Roundish colonies, at first with perfectly even borders, later sinuously broken, not unlike liquefying sarcina colonies. With accurate focusing the form of tetrads is recognizable in the gray, transparent peripheral portion; toward the center the colony is opaque, shaded gray. *Deep:* Irregularly formed, smooth border, opaque, delicately to coarsely granular (7, VIII).

Gelatin Stab.—*Stab:* At first thread-like, later, in upper part very granular, in lower part like a string of pearls, white (7, II). *Surface growth:* After ten days from 3 mm. to 4 mm. broad, irregularly round, partially lobulated, much elevated in the center, like the head of a nail, moist, pure white or somewhat yellowish, shining (7, III).

Agar Plate.—The same as upon gelatin, only much more luxuriant, opaque (7, VI).

Agar Stab.—*Stab:* Confluent, very rough, pure white. In old cultures there often occur luxuriant outgrowths in clumps (7, V). *Surface growth:* Irregularly round, sinuous or wavy. Much elevated, often with terrace-like formation, pure white, with an oily luster, at times with a suggestion of yellow (7, IV). The agar streak corresponds. Water of condensation clear, with white precipitate (7, I).

Bouillon Culture.—Clear; moderate precipitate, upon shaking becoming distributed at first as flocculi and then homogeneously.

Milk Culture.—After four days firmly coagulated, at other times coagulation is absent.

Potato Culture.—Limited to the streak of the inoculation, sharply outlined from the surroundings, but not elevated. Border of the growth irregular and jagged, pure white, dull, or faintly shining. According to Gaffky, thick, slimy, tenacious (7, X).

Chemical Activities.—It produces some acid upon grape-sugar bouillon, and a strikingly strong odor of glue upon agar plates. It does not liquefy gelatin, nor does it form H_2S or indol upon 2% solution of peptone.

Distribution.—(*a*) *Outside the organism:* We have never met it.

(*b*) *In the healthy organism:* In the mouth; found by Boutron in human milk.

(*c*) *In diseased human organism:* In pulmonary cavities in phthisis (Gaffky); in abscesses.

(*d*) *In animals:* Found as cause of suppuration several times (Karlinski, C. B. VII, 113).

Experimental Observations Regarding Pathogenic Effects.—(*a*) *Upon animals:* In white mice it causes a rapidly progressing septicemia. Guinea-pigs and white rats are similarly susceptible. In rabbits there is usually only a local affection (peritonitis, abscess, etc.). Gray rats and gray mice are very resistant or even immune.

(*b*) *In man:* It has been demonstrated that the organism causes suppuration and not merely accompanies it (Viquerat, Z. H. XVIII, 411).

Special Methods of Detection.—Agar plates, microscopic picture, experiment on the mouse. In bouillon and hay decoction no packets of sarcina are formed.

Morphologically identical but not pathogenic is the **Micr. tetragenus albus** Boutron. The **Micr. tetragenus aureus** Boutron is liquefying, non-pathogenic, and was grown from human milk. It was observed by Boschi and Bellei (C. B. XXIII, 856), after repeated growth, to become colorless. They very properly consider all these forms as only varieties of the Micr. tetragenus.

Related Varieties.

We are unacquainted with the **Micr. tetragenus subflavus,** obtained by Besser from nasal mucus, which did not grow upon gelatin, and was yellowish upon agar (Ziegler's "Beiträge zur path. Anat.," VI, 347).

We are unable to differentiate the **Actinobacter polymorphus** Duclaux by means of a culture from Král.

The **Micr. tetragenus mobilis ventriculi** Mendoza (C. B. VI, 566) is theoretically interesting. From the description it cannot be differentiated from the Micr. tetragenus Gaffky and Koch, except that it forms some skatol. It possesses a very lively spontaneous motion, and constitutes, until more material is at hand, the motile, presumably flagellated, related form of the Micr. tetragenus (see Micrococcus roseus).

Micrococcus rosettaceus (Zimmermann) (i, p. 72).

According to the description of Zimmermann it is almost identical with the Micr. candicans, but upon gelatin it is grayish-white and upon potato of a yellowish-gray color ; size from 0.7 μ to 1.0 μ.

Micrococcus concentricus (Zimmermann) (i, p. 86).

Upon all nutrient media it forms only a thin, delicate, iridescent growth, resembling somewhat the Bact. typhi. Upon gelatin plates the border is irregular, and concentric zones are almost always seen on gelatin. It never liquefies gelatin. Upon potato thin, yellowish-gray, slimy growth. They are 0.9 μ in diameter. Found by Zimmermann in Chemnitz hydrant-water.

Micrococcus viticulosus (Katz).

This was, so far as we know, isolated only once by Katz in Flügge's laboratory in Göttingen. In the gelatin stab and in deep colonies in gelatin plates it forms delicate white tendrils. We know it only from the description, according to which its cultures evidently have a great similarity to those of the Bact. Zopfii, which we have represented in Plates 29 and 30. Gelatin is not liquefied. The cocci are always oval, being 1.2 μ long and 1 μ broad.

Micrococcus of Bitter Milk (Conn) (C. B. ix, 653).

Rather large coccus, non-chromogenic. Gelatin rapidly liquefied, which, as well as bouillon, becomes very mucilaginous. Milk at first is coagulated, then becomes clear and slimy. It tastes faintly acid, but is very bitter.

Micrococcus Freudenreichii (Guillebeau).

Large cocci (2 μ and over in diameter), usually single, rarely (in bouillon) arranged in chains. In milk gelatin, the colonies first appear white, entire, finely granular; after two days rapid liquefaction occurs. Agar culture is white. Potato culture sulphur-yellow to yellowish-brown, at times delicate and at other times luxuriant. Bouillon first mes cloudy, then clear with a flocculent sediment. In sterile milk there is formation of acid, early there is marked stickiness (tenacity[1]), after a few days coagulation. Optimum 20°. Growth occurs from 11° to 35°. It is perhaps a streptococcus.

Micrococcus acidi lactis (Krüger) (C. B. vii, 425, 464, 493).

Oval coccus, forming diplococci and tetrads, from 1.0 μ to 1.5 μ in diameter. It is a facultative anaerobe. Round, white colonies in gelatin, with ragged border. Gelatin is liquefied. *Gelatin stab. Stab:* Granular, white growth. *Surface growth:* White and later sinking downward. From milk-sugar it forms lactic acid. Milk is coagulated in five days at from 15° to 35°, then the albuminous bodies are peptonized with the production of a sticky character and a pasty odor.

Micrococcus coronatus (Flügge) (Ed. iii, p. 178).

Round cocci, from 0.8 μ to 1.6 μ. *Gelatin plate. Natural size:* At first small, white disks, which, when they are on the surface, have a broad zone of liquefaction. At this stage, if magnified sixty times, the colonies appear as gray, coarsely granular disks, with ragged borders, and later they break up into fragments and crumbs. The picture of the natural-sized colonies later is quite changed; while a yellowish-white, irregular clump lies at the bottom of the shallow funnel of liquefaction, the clear funnel of liquid is surrounded by a zone of sturdy, irregular points and outgrowths, which make the picture very striking. The *gelatin stab culture* resembles that in the plate.

Agar plate: Deep colonies, round, white, almost opaque. Superficial, at first round, then ragged, lobulated, wavy, luxuriantly developed. *Agar streak:* Grayish-white, broad, jagged, somewhat dry. *Potato culture:* Similar. *Bouillon:* Slightly cloudy with sediment. No indol, a trace of H_2S is formed. *Milk* becomes gelatinous in ten days; after fourteen days it is coagulated with a minimal acid reaction.

Found by Flügge many times in examinations of air, by us in examination of smegma.

Micrococcus corallioides (Zimmermann) (ii, p. 72).

According to Zimmermann's description it resembles the preceding, yet is entirely different. *Gelatin plate. Natural size:* Colonies appear as white, somewhat irregular masses, which after eighty hours form outgrowths all around, so that finally they lie in the half liquefied gelatin, with radiating, often branched, formations extending in all directions. Magnified one hundred times the masses of bacteria appear granular. Also the milk-white growth at the top of the gelatin stab sends out indistinct outgrowths. Upon agar a broad, milk-white growth, upon potato very little growth. Meat-infusion is uniformly cloudy. Found by Zimmermann in water.

[1] Weigmann's Micr. of tenacious milk does not liquefy gelatin.

Micrococcus radiatus (Flügge) (Ed. iii, p. 179).

Micrococci of less than 1 μ. The deep colonies in the gelatin plate, which are at first granular and sharply outlined, when they come to the surface become surrounded by a ring of pretty rays, which separate a little at the periphery, so that the colony is somewhat irregularly outlined. Later second and third rings of rays may develop. In the gelatin stab culture there is a pointed funnel of liquefaction. From the lower part of the stab horizontal outgrowths radiate, so that the stab appears as if feathered. We have not seen it; description is from Flügge. The color is described by Flügge as white, with a yellowish-green shimmer.

Micrococcus luteus (Lehm. and Neum.).

(Plate 6, I–V.)

Synonyms.—The insufficiently defined Micrococcus luteus Cohn, designated by Schröter as Bacteridium luteum, is not to be identified as a particular variety. We designate the species to be described in this way, to express the relation to the Sarcina lutea.

Microscopic Appearance.—Medium sized (0.4 μ to 1.2 μ), roundish cocci, often lying together in fours, often only in pairs.

Relation to Oxygen.—In shake cultures strongly aerobic.

Requirements as to Temperature and Nutrient Media.—Grows rapidly and luxuriantly at room and incubator temperature upon all nutrient media.

Gelatin Plate.—(*a*) *Natural size:* Yellowish to yellowish-white irregularly round colonies, after three days from 1½ mm. to 2 mm. broad. In a short time they sink in, without the form of the colonies being disturbed. Later there follows a breaking up of the film into irregular granules and debris.

(*b*) *Magnified fifty times. Superficial:* Yellowish-gray to grayish-brown, irregular, roundish colonies with wavy, scalloped borders. In the periphery, at times individual tetrads are plainly visible. The outer part is more transparent than the central portion. The interior is uniformly shaded in a gray color. *Deep:* Roundish to whetstone-shaped, smooth border, finely granular, of the same color as the superficial (6, II).

Gelatin Stab.—*Stab:* Until liquefaction begins it is granular. After two days liquefaction begins with a plate-shaped depression, which later becomes cylindric. The *contents of the funnel* are cloudy, greenish or yellowish-gray (6, 1).

Agar Plate.—Both when of natural size and when magnified fifty times, the colonies are like those in the gelatin plate, only the granulation is finer. Sometimes there are found thin, pale yellow, transparent deep colonies, as much as 2 mm. broad, with a coarsely granular or morulated structure.

Agar Stab.—*Stab:* Granular, yellow. *Surface-growth:* Lemon-yellow, shining, roundish, with wavy border, somewhat elevated.

Agar Streak.—Corresponding to the stab. Water of condensation clear, sediment yellowish.

Bouillon Culture.—Remains clear. The yellowish precipitate is closely packed, rising up tenaciously only with energetic shaking, and afterward becoming homogeneously divided.

Milk Culture.—After twenty days it is half coagulated. Acid reaction.

Potato Culture.—Lemon-yellow to yellowish-green, thin, faintly shining, with a wavy, irregular border, and almost no elevation whatever. Sharply outlined from the surroundings.

Related Varieties.

We consider this form identical with sarcina lutea—only our form produces no sarcina groups, either upon solid nutrient media, or in bouillon, or even in hay infusion. Sarcina lutea would be its "sarcina form."

The **Streptococcus liquefaciens** and **Pediococcus flavus**,[1] obtained from Král, are identical. The Strept. liquefaciens only produces a cloudiness of bouillon and of the funnel of liquefied gelatin, and in old agar streaks has a brownish-yellow shade—variations which are constantly observed in Micr. pyogenes α aureus. From the description the **Micr. galbanatus** Zimmermann is also identical, and, as we saw subsequently, was found by Zimmermann to be identical with the

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[1] Recently we have discovered beautiful packets of sarcina from old hay infusion cultures of Pedioc. flavus. These were not so evident in Strept. liquefaciens.

Strept. liquefaciens Král. One should ordinarily give the unambiguous name of Zimmermann the preference over Micr. luteus, yet we desired to indicate the analogy to the Sarc. lutea.

Micrococcus flavus (Flügge). Lehm. and Neum.

Completely identical with the former, only it has *finely granular* gelatin colonies and less tendency to the formation of tetrads. We consider this form identical with the already described Sarc. flava, with which it corresponds, with the exception of the ability to produce sarcina packets. We have obtained this organism as **Staphylococcus citreus** from C. Fränkel and as Sarc. flava from Prague. The latter was always without sarcina packets. We have been unable also to differentiate what we obtained as **Micr. citreus agilis** Menge (C. B. XII, 49). It is devoid of flagella, very weakly liquefying, and non-motile.

There appear to be transitions from the Micr. flavus to the Micr. luteus.

Micrococcus sulfureus Zimm., Elaborated by Lehm. and Neum.

We cover, provisionally, with this name all lemon-yellow as well as greenish to grayish-yellow cocci, which do not liquefy gelatin, of which we have cultivated many from the air and water. They were all *finely granular* upon gelatin plates. We consider them as non-liquefying forms of Micr. flavus L. and N.[1] Here also belongs the **Micr. sordidus** Schröter.

Micrococcus sulfureus β tardigradus (Flügge). (Lehm. and Neum.)

Micrococcus flavus tardigradus (Flügge), page 178.

It is differentiated from the former only by very slow growth. Found by Zimmermann in water. Only a variety of the former. Once we found a Micr. sulfureus in the air whose superficial colonies produced sometimes no, sometimes very little, and again very active, liquefaction, thus being a transition to the Micr. flavus.

Micrococcus badius (Lehm. and Neum.).

Medium-sized, round cocci, often united in tetrads, never showing sarcina forms upon any nutrient medium. In the *gelatin plate* the colonies appear as glue-brown, slightly elevated, transparent drops, which when magnified sixty times appear entirely homogeneous or at most with concentric zones. *Agar plate* is similar. *Gelatin stab:* a not very luxuriant, glue-brown, shining growth ; along the stab a delicately granular growth. *Agar stab:* Moist, transparent, glue-brown. Gelatin is very slowly and slightly liquefied. Bouillon uniformly cloudy. Upon potato there occurs a dark yellowish-brown, gelatinous growth. Growth always slight. No growth in milk.

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[1] We have never found coarsely granular, non-liquefying forms resembling the Micr. luteus.

It was obtained from Král as Sarc. lutea, and was not met by us elsewhere. It reminds one of the Sarc. fulva Stubenrath.

Micrococcus ascoformans[1] (Johne).

Synonyms.—Discomyces equi Rivolta, Micr. botryogenes Rabe, Botryomyces Bollinger, Botryococcus ascoformans Kitt.

Literature.—Kitt (C. B. III, 177), Schneidemühl (C. B. XXIV, 271).

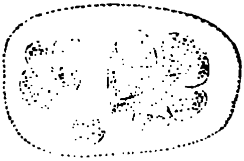

Fig. 15.—Ascococcus Billrothii Cohn (after F. Cohn).

It occurs in the tissue and pus of the pathologic formation, grouped like grains of sand, surrounded by a gelatinous mass with a double-contoured, shining covering. In cultures no capsule is formed, except that upon blood-serum hartshorn-like plugs occur.

According to Johne's description the cultures very much resemble those of the Micr. luteus and flavus. The micrococci are usually arranged in pairs or fours. *Gelatin plate:*

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[1] The Micr. ascoformans recalls involuntarily an organism which Cohn had described as Ascococcus Billrothii. It forms spherical or lobulated colonies upon artificial nutrient media, which possess a thick, gelatinous or cartilaginous capsule. A similar organism was described by Hankin as Ascococcus cantabridgensis, obtained from the mouth of a student in Cambridge. The coccus quickly covers agar with a transparent, slimy, very delicate covering of yellowish-white color, and grows rather slowly in bouillon and gelatin. It is different from Asc. Billrothii in the oblong form of its individual groups and the less distinctly visible capsule.

Macroscopically they appear as if sprinkled with grayish-yellow pollen, with a fruit-like odor.[1] *Magnified sixty times:* Round, sharply outlined colonies without special characteristics. In the gelatin stab culture liquefaction takes place slowly, with a cup-shaped depression. The growth along the stab is white and thread-like. Upon *potato* a hoarfrost-like yellowish deposit with a fruit-like odor. Upon *agar* the growth is scarcely perceptible. Kitt has expressed the belief that the organism is only a special form of the Micr. pyogenes—which requires further investigation.

It is pathogenic for guinea-pigs, sheep, goats, cows, swine, and especially for *horses*. It is found in thick, cord-like or nodular connective-tissue growth, usually softened in the center, in the perimysium, subcutis, spermatic cord (after castration) and the retroperitoneal connective tissue of horses. Besides, it is found in the lungs, udder, lymph-glands, ear muscles, nasal mucous membrane and bones. Recently cases have been described also where botryomycosis occurred in man (compare Schneidemühl, *l. c.*).

Micrococcus pyogenes (Rosenbach) (Lehm. and Neum.).

(Plates 8 and 9, I–III.)

α Aureus (Rosenbach) Lehm and Neum.
β Citreus (Passet) " " "
γ Albus (Rosenbach) " " "

Synonyms.—Staphylococcus pyogenes aureus Rosenbach, Staph. pyogenes albus Ros., Staph. pyogenes citreus Passet.[2]

Ordinary Names.—Grape coccus, pus coccus, simply "staphylococcus."

Principal Literature.—Rosenbach, "Mikroorganismen bei den Wundinfektionskrankheiten des Menschen," 1884; Passet, "Aetiologie der eitrigen Phlegmone," 1885; Garré, "Fortsch. d. Medic.,"

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[1] Our Micr. luteus also possesses a sometimes agreeable sometimes disagreeable sweetish odor.

[2] Compare also p. 187, regarding the Staphylococcus citreus Passet.

1885, III, 165; Lübbert, "Biologische Untersuchungen über den Staph. pyog. aureus," Würzburg, 1886.

Introductory Remarks.—For the comprehension of the three forms given above as varieties of one form certain proof was hitherto lacking. R. O. Neumann (A. H. XXX, 1) furnished it when he observed that in orange-colored colonies sometimes lighter white or yellow sectors appear (similar to those in Micr. bicolor), and by inoculation from these, cultures are obtained which still more markedly present the formation of paler sectors. By repeated consistent transfers in this way white and yellow cultures can be grown from orange-colored cultures, and even a red culture could be obtained. These new cultures remain in part permanent and in part revert to the original form. Also consult Neumann concerning what was otherwise known regarding the variations of this form.

Highly probable synonyms: **Micrococcus liquefaciens conjunctivæ** Gombert, Eisenberg, 301; **Micrococcus flavus conjunctivæ** Gombert, Eisenberg, 302; **Staphylococcus salivarius pyogenes** Biondi, Eisenberg, 309.

The effort of various authors to found a specific differentiation of the three forms upon varying virulence is wrong. In the first place, the fact that the golden-yellow form is distinguished by special virulence (v. Tavel, Lannelongue, and Achard) is disputed. Levy found that the more common form in Strassburg was the white form, and it was just as pathogenic. In the second place, it is easily shown experimentally that enormous reduction of virulence is entirely independent of the color (compare page 185). Growth without oxygen which increases the virulence lessens the production of pigment.

In the following the **Micr. pyogenes** α **aureus** only is particularly described. Regarding the β citreus and γ albus see page 187.

Microscopic Appearance.—Round, smaller or larger cocci, on an average 0.8 μ, in pairs or singly, usually in grape-like clusters. Often they have a small division cleft (8, X and XI).

Relation to Oxygen.—Grow well aerobically and not so well anaerobically.

Requirements as Regards Temperature and Nutrient Media.—Optimum at 37°, but grows well at room temperature; thrives upon all nutrient media, the pigment is developed most abundantly upon agar and potato.

Gelatin plate. (*a*) *Natural size:* Small, irregularly roundish colonies of yellowish-white to yellow color. After six days, 1½ mm. in diameter. Old colonies are not much larger. The colonies usually sink slowly into the medium and become surrounded by a flat, plate-like zone of liquefaction (8, VII).

(*b*) *Magnified seventy times. Superficial colonies:* Roundish, faintly yellow to brown, with delicate, transparent peripheral zone. Structure somewhat coarsely granular, toward the periphery a little more finely granular (8, VIII). *Deep colonies:* Roundish to whetstone-shaped, dark yellow to brown, structure finely granular, border almost smooth.

Gelatin Stab.—Liquefaction along the line of puncture after two to three days. The zone of liquefaction is conical to bag-shaped, and later cylindric. The contents of the cavity are grayish-white, cloudy in appearance, and at the bottom a whitish to orange-yellow pigment is deposited in little clumps. The intensity of the liquefaction varies widely.

Agar Plate.—(*a*) *Natural size:* The *superficial* colonies are round or roundish, orange-yellow, faintly shining, evenly elevated, and as much as 4 mm. in diameter. *Deep:* Roundish to whetstone-shaped, equally or more deeply colored and never so large as the superficial (8, V).

(*b*) *Magnified sixty times. Superficial colonies:* Round, almost or entirely even border, with transparent, delicately punctated peripheral zone, orange-yellow, toward the center shaded to homogeneous gray, sometimes with a more darkly colored ring near the periphery. *Deep:* Colonies partly roundish, partly whetstone-shaped, dark grayish-yellow, opaque, at periphery often somewhat more coarsely granular. Often there are found in agar, broad, pale yellowish, round, transparent colonies with the granulation more marked (8, VI).

Agar Stab.—*Stab:* Insignificant growth, at first thread-like, later slightly granular. *Surface growth:* Roundish, evenly elevated, with smooth, somewhat wavy border, faintly shining, orange-yellow (8, III).

Agar Streak.—Corresponds to growth in the stab.

Water of condensation cloudy. Precipitate whitish-orange (8, II).

Bouillon Culture.—Marked uniform cloudiness. On the surface a delicate pellicle is formed. Sediment moderate and upon agitation it breaks up into tiny flocculi. In sugar bouillon the same.

Milk Culture.—According to Passet's and our own observations it is gelatinous or firmly coagulated in from one to eight days. According to Tavel it is flaky.

Potato Culture.—Limited to the streak, at first whitish, later pale orange-yellow, somewhat elevated and crumbly, shining. Old cultures are wider, deep orange, and dry (8, IX).

Viability and Resistance.—(*a*) *In body:* Many cases in which the Micr. pyogenes has been found after very long periods (from ten to thirty-five years), in areas (osteomyelitis) where they had been encapsulated during this time, appear to prove a long viability.

(*b*) *In cultures:* Very tenacious of life. Even after many months are always alive, still there are no resting forms.

Resistance to—(*a*) *Drying:* According to Hägler, they are alive after from fifty-six to one hundred days in dried pus.

(*b*) *Dry heat:* According to Lübbert, they are killed in one hour at 80°, and killed rapidly only at from 110° to 120°.

(*c*) *Moist heat:* Seventy degrees kills very quickly.

(*d*) *Cold:* Are alive after sixty-six days in ice (Prudden).

(*e*) *Disinfecting agents* act rather slowly. In bouillon cultures $\frac{1}{1000}$ sublimate does not kill them within five minutes.

Chemical Activities.—(*a*) *Pigment production:* Produces orange-yellow pigment of the carotin group (compare p. 66), but only if oxygen is admitted. According to Lübbert and F. Gärtner, pigment production is greater, the more oxygen is contained in the air.

(*b*) *Substances that are smelt or tasted:* Agar cultures smell like glue or spoiled yeast or paste (Becker, Passet).

(*c*) *Gas and acid formation from carbohydrates:*

Rather abundant formation of acid from grape- and milk-sugar, but no gas-formation. It forms from milk-sugar: lactic acid and volatile fatty acid ; from dextrose : lactic, acetic, and valerianic acids ; from glycerin : lactic, isobutyric, valerianic, and propionic acids (Terni).

(*d*) *Sulphuretted hydrogen:* Rapid and abundant.

(*e*) *Indol:* Only a little.

(*f*) Sometimes it produces a vigorous fermentation of *urea* (Barlow, Mann). Other cultures are almost without effect upon urea.

(*g*) *Poisons:* See under animal experiments.

Distribution.—(*a*) *Outside the animal organism:* In milk, wash-water, dirty water (little in pure water and soil), and air it is widely distributed. The Micr. pyogenes makes up 10% of the micro-organisms found in the air of surgical operation room (compare Ullmann, Z. H. IV, 55).

(*b*) *In healthy body:* Upon the skin, especially of the head, in the mouth and vagina, and not uncommonly in the cervix uteri. Also found in the milk of healthy women.

(*c*) *In diseased human organism:* All processes accompanied by suppuration or only inflammation in the various regions of the body may be caused by staphylococci, and are thus caused in a large percentage of cases. In other cases they act together with the Strept. pyogenes, Strept. lanceolatus, Bact. coli, Bact. typhi, etc. It must, however, always be firmly maintained that the last-named organisms (as well as some others) may alone equally well cause suppuration.

The following affections especially are often dependent upon staphylococci : Acne of the sebaceous glands, sycosis of the hair follicles, hydradenitis of the sweat glands, pemphigus,[1] phlegmon, furuncle, abscess, periostitis, osteomyelitis, septicopyemia.[2]

They rarely cause erysipelas (Jordan). Fibrinous inflammation may also be caused by them (Guthmann). Gradenigo and Maggiora observed croupous inflammation of the nasal mucous membrane caused by staphylococci (C. B. VIII, 641).

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[1] Consult also page 188.

[2] Sahli has demonstrated it in the joints in a case of articular rheumatism. Singer has constantly cultivated staphylococci or (more rarely) streptococci from the urine in seventeen cases of severe and mild articular rheumatism. The organisms were abundant during the disease and disappeared with recovery (C. B. XVIII, 130).

The following inflammations are also produced by the Micr. pyogenes, but more rarely than by other varieties, as Strept. lanceolatus, Strept. pyogenes, etc. : pleuritis and pericarditis, pneumonia, hepatitis, etc.

(*d*) *In diseased animal organism :* Just as in man as the cause of suppuration. Statements that animals have other causes of suppuration than man are erroneous. It caused an epidemic osteomyelitis in geese (Lucet) and a disease of gudgeon (Charrin) in France.

Experimental Observations Regarding Pathogenic Effects.—There is an extraordinary variation in the disposition of different apparently identical experimental animals, as also in the virulence of the micro-organisms themselves.

The disposition is greater in young, anemic and diabetic animals. The virulence of micro-organisms freshly obtained from an animal or man is often considerable, but sometimes even in this case it is slight. By growth upon our artificial nutrient media it is sometimes rapidly reduced and sometimes almost imperceptibly changed. By repeated passage from animal to animal, in fatal doses, the virulence is increased for the concerned species (Terni), also simultaneous inoculation with other bacteria (Ortolani and De Blasi) or their metabolic products (for example, the Bact. vulgare) increases the virulence. In the same way repeated anaerobic cultivation heightens the virulence. The intensity of the liquefaction of gelatin is almost, but not entirely, parallel with the pathogenic property.

With the most exalted virulence the staphylococcus causes no local suppuration, but a gelatinous edema, hemorrhages in the kidneys, often inflammatory changes in the cardiac valves and in the aorta. The relative susceptibility of animals to infection with staphylococci is shown in the following decreasing series : Horse, dog, man, cattle, sheep, rabbits, guinea-pigs, mice. In the last-named numerous bacteria are necessary for infection.

Subcutaneous injections cause abscesses, but a rather large number of germs (according to Herman, 50,000,000 individuals equal 1 c.c. of culture) from a not highly virulent culture is required to do this in rabbits.

Intraperitoneally large quantities (13 c.c. and more) are borne by rabbits; infection of the pleura and surface of the lung has often been undertaken.

Intravenous injection causes endocarditis, especially after previous injury of the valves of the heart, and usually also nephritis. The hyperemic kidney shows, macroscopically, yellowish, wedge-shaped areas in the cortex, in which the straight urinary tubules are filled partly with casts partly with cocci, and some are empty and compressed. Cocci are found in the urine.

Injection into joints causes suppuration.

In man acne, furuncles, and phlegmons have been produced by rubbing into the skin.

Toxins, Immunity and Immunization.—The filtered bouillon culture contains toxic substances which act intensely. Injection of this into the peritoneal cavity of dogs causes a sero-bloody peritonitis, ecchymoses in the serosa and mucous membrane of the intestine, and death from bloody diarrhea. By suitable modification of the toxic property, quantity, etc., of the injected metabolic products Kraft could produce all the forms of typical peritonitis.

Subcutaneous injection of the filtered bouillon culture produces all the processes, from a doughy swelling, which disappears without suppuration, to typical suppuration, even to fibrino-hemorrhagic necrotic inflammation. This depends upon the virulence of the bacteria employed.

According to Viquerat (Z. f. B. XVIII, 487), the bouillon culture contains no specific poisons, only pyogenic bodies, which are widely distributed.

After repeated injections of sterilized or filtered cultures different authors have obtained entirely different results. We quote only two examples:

Reichel observed a greater or less resistance to intraperitoneal injection of the staphylococcus poisons in animals which he had injected intraperitoneally for a long time at intervals of from two to five days with filtered or otherwise sterilized bouillon or gelatin culture, and even a relative immunity against the pus cocci themselves. Nannotti observed only chronic intoxication and no immunization in animals which had received many injections of the metabolic products.

According to Rodet and Courmont, this contradiction is explained by the simultaneous presence, but in varying proportion, of an immunizing and a predisposing substance, the former being precipitated and the latter soluble in alcohol. Tavel was, however, unable to produce immunity with the alcoholic precipitate, the animals either dying from chronic intoxication or succumbing to an additional injection with virulent cocci.

The *serum* of actively immunized animals has no noticeable effect upon the Micr. pyogenes in vitro; it is also, so far, of scarcely any practical value for producing passive immunity.

Special Culture Methods.—Isolation is accomplished most rapidly by means of agar plates at incubator temperature. The potato culture is best for the study of the chromogenesis. Milk cultures and animal investigations are necessary.

Micrococcus pyogenes γ albus (Rosenbach).

In all respects like the Micr. pyogenes α aureus. See Plate 9, I and II, and the remarks on page 181.

Here belongs the Micr. ureæ liquefaciens Flügge (compare page 71).

Micrococcus pyogenes β citreus (Passet).

We have studied this organism only in a culture obtained from C. Fränkel, and designated by him as identical with the Micr. flavus (page 178). It did not coagulate milk and produced a slow liquefaction of gelatin with formation of air-bubbles. A Micr. pyogenes citreus is said to exist, however, which corresponds entirely with the Micr. pyogenes aureus except in the color. With this the results of cultures by R. O. Neumann agree (page 181).

Varieties Closely Related to or Identical with the Micrococcus pyogenes Ros. (Lehm. and Neum.).[1]

Micrococci in Variola.

Vanselow and Czaplewski (Vierteljahrsschr. f. gerichtl. Med., 1899, Heft 1) believed they had found an organism closely connected with the variola process in what was previously named by Klebs the Micr.

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[1] The old names, **Staphylococcus cereus flavus** and **Staph. cereus albus** Passet, can not be sharply defined, and can well be dispensed with. These varieties are rarely cultivated from pus and grow upon

quadrigeminus Klebs. It was very like the Micr. pyogenes albus. (However, it liquefied solidified blood-serum, which the typical Micr. pyogenes is said not to do; its color is reddish, but in this property is variable, as is to be expected.) They have already retracted this hardly probable suspicion (C. B. xxv, 546).

Almost simultaneously Sanfelice and Malato (C. B. xxv, 641) have reported that a coccus can be constantly cultivated from cases of variola, which can not be differentiated morphologically from the Micr. α aureus, but differs in its pathologic action from all other cultures of Micr. pyogenes isolated by the authors. When injected into the circulation, hyperemia of the skin and mucous membrane and sharply outlined hemorrhages occur.

Regarding the much controverted "Cytoryctes variolæ" Guarnieris, of the group of protozoa, consult the literature in Galli-Valerio, Kritische Uebersicht über den Zusammenhang der Variola mit Vaccine (C. B. xxv, 380 and 424).

Staphylococcus pemphigi neonatorum Almquist[1] (Z. H. x).

According to Strelitz (C. B. xiii, 107), the Micr. pyogenes is itself the cause of pemphigus, and besides being cultivated from pemphigus vesicles, is able to reproduce the condition. Others obtained similar results; for example, Bodenstab (compare Vogel) found that four children cared for by the same midwife developed pemphigus within two weeks (C. B. xxi, 288).

Micrococcus biskra Heydenreich.

(Cause of the Pende's ulcer, tropical ulcer, Delhi boil, Clou de Biskra, etc.)

According to the description of Heydenreich, it can not be differentiated from the Micr. pyog. α aureus (C. B. v, 163). The statement by Chantemesse (C. B. v, 221) that the gelatin is very slowly liquefied also applies to many cultures of Micr. pyogenes. Chantemesse gives as other points for differentiation from the Micr. pyogenes, its whitish growth upon agar, and its luxuriant, rapid, watery and orange-red

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the surface in the gelatin stab as a faintly shining, waxy deposit with a somewhat thick border. Both varieties are closely related to the Micr. β citreus and γ albus. They often pass as forms of these, but are differentiated, according to the insufficient description at hand, by absence of liquefaction and slight or no pathogenic quality.

Without being able to show this interpretation to be incorrect, we refer to our note (p. 170) that the Micr. cereus albus was found by us to be identical with the Micr. candicans Flügge, with the exception of its smaller size. We are not familiar with the Micr. cereus flavus; it may perhaps belong to Micr. sulfureus Zimmermann.

[1] The Dipl. pemphigi acuti Demme, appears different. Is grown only at incubator temperature (Cong. inn. Med. Wiesbaden, 1886).

growth upon potato. These characteristics are not sufficient for separating it, especially as Heydenreich does not describe his potato cultures as essentially different from those of the Micr. pyogenes. Raptschewsky declares (C. B. VI, 504) the Micr. biskra identical with the Micr. pyogenes, and prefers to consider a streptococcus as the cause of the disease.

Micrococcus of gangrenous mastitis in sheep, Nocard (A. P. I 417). Staphylococcus hæmorrhagicus, E. Klein (C. B. XXII, 81).

De Jong's **Staphylococcus bovis** is said to be different from the Micr. pyogenes. Injected subcutaneously, intraperitoneally, and intravenously it is pathogenic for rabbits, dogs, and guinea-pigs. Neither its white nor its yellow form liquefies gelatin, in spite of luxuriant growth; milk is not coagulated; in bouillon it forms a delicate, tenacious sediment.

The cause of a circumscribed falling of hair, without discoloration of the hair-bed and without a tendency to spread, is found, according to Vaillard and Vincent, in a white liquefying coccus, 1 μ in diameter, which corresponds throughout, in its growth, to the Micr. pyogenes γ albus (A. P. IV, 1890, 446).

(Literature by Hollborn, C. B. XVIII, 47, 108.)

Micrococcus bicolor (Zimmermann).

Round cocci from 1.2 μ to 1.6 μ. *Gelatin plate:* At first yellowish, succulent, elevated; later, orange-yellow, slowly sinking, oily looking colonies of round form; besides these there are others about the same, but white in color. Magnified sixty times they are even-bordered and faintly granular. *Gelatin stab:* Superficial growth is white, with a slowly forming cup-shaped liquefaction. The growth along the stab is thread-like. *Agar plate* is like gelatin, and also presents gray and yellow colonies intermixed. *Agar streak:* Succulent, whitish or grayish-yellow growth with orange-yellow islands and points. The surface growth in the agar stab always presents more or less perfect gray and orange sectors, from which it is often possible to obtain pure gray or pure orange-colored growths, but which in following generations again produce the two colors. *Bouillon* becomes diffusely cloudy with moderate, firm precipitate. Milk becomes a little acid and remains fluid. Upon 2% peptone bouillon it forms a trace of H_2S and indol. We have obtained this organism, which was isolated by Zimmermann from tap-water, from gastric contents. The **Micr. cremoides** Zimmermann is very closely related to this. We were entirely unable to differentiate the culture obtained by Zimmermann.

Also, the **Micr. aurantiacus** Cohn, which we obtained from Král, is distinguished only by the absence of liquefaction. We have also obtained from it white, orange, and striped cultures, which pass from one into the other.

At present we can give no other decisive characteristics of the Micr. bicolor, aurantiacus, and even of the Micr. candicans as differing from the Micr. pyogenes except the pathogenic action in animals and absence of liquefaction.

Micr. roseus (Bumm) (Lehm. and Neum.).

(Plate 11.)

Synonyms.—Diplococcus roseus (Bumm) Flügge. See end of section.

Microscopic Appearance.—Round to irregularly roundish cocci (from 0.6 μ to 1.0 μ), often with rather wide line of division in the cocci (11, XIII), at other times more complete cocci lie together in pairs and small groups.

Motility is lacking. Compare page 192.

Relation to Oxygen, Nutrient Media, and Temperature.—Grows slowly upon all nutrient media, best at room temperature, also at 37°. In shake cultures it grows only near the surface, the deep colonies only very slightly. Pigment is only produced when air is admitted.

Gelatin Plate.—(*a*) *Natural size: Superficial*, irregularly roundish, small, rose-red. After a long time they become somewhat larger, evenly elevated, shining. The *deep colonies* grow very little. After weeks the superficial ones sink gradually into the gelatin.

(*b*) *Magnified fifty times:* Round or roundish colonies, almost even borders, rather finely granular, colored pale to rose-red. The deep appear the same, only they are smaller (11, VII).

Gelatin Stab.—*Stab:* Thread-like. After several weeks the gelatin begins to liquefy in a cylindric form. After three months the growth has sunk in about 1 cm. *Surface appearance:* Roundish, sometimes lobed, rose-red growth, which, later, on account of the liquefaction of the gelatin, is almost entirely lost (11, I).

Agar Plate.—(*a*) *Natural size:* Like gelatin.

(*b*) *Magnified fifty times. Superficial:* Round or roundish colonies with even or somewhat wavy border, yellowish to red, from the most delicately punctated to coarsely granular (11, V), transparent, more intensely colored toward the center. *Deep:* Roundish to whetstone-shaped, border smooth or granular, finely to coarsely granular (11, V, VI), opaque, darker than the superficial in color.

Agar Stab.—*Stab canal:* Thread-like, later granular (11, III). *Surface growth:* Roundish, evenly elevated, oily, rose-red, of the consistency of butter (11, IV).

Agar Streak.—Growth spreads little, smooth border, wavy. Water of condensation clear, reddish sediment (11, II).

Bouillon Culture.—Clear (only rarely more or less cloudy). Sediment reddish, abundant, and coherent.

Milk Culture.—Usually unchanged.

Potato Culture.—Limited to streak, faint rose, with oily luster, somewhat elevated, often surrounded by a whitish, glistening zone (11, X).

Special Nutrient Media.—If the Micr. roseus is grown upon the culture of a representative of the subtilis or anthrax group its colonies grow considerably more luxuriantly and take on a more intense color (11, IX). (Doubtless on account of the alkalinity of the potato.)

Distribution.—(*a*) *Outside the body:* Very common and widely distributed air-organism, scarcely ever absent from a plate from the air in Würzburg.

(*b*) *Inside the body:* Not demonstrated.

We have closely compared this fungus—which, I believe, was primarily described from Würzburg as "rose-colored diplococcus" of Bumm—with the following imported varieties:

1. Micr. agilis Ali-Cohen, isolated by Prof. Zimmermann in Chemnitz.
2. Micrococcus agilis Ali-Cohen, hygienic institute in Berlin.
3. Micrococcus roseus (author?) from Prof. A. Fischer in Leipzig.
4. Micrococcus tetragenus ruber. From Král in Prague.
5. Staphylococcus roseus Tavel. From Prof. Tavel in Bern.
6, 7, 8, 9. Four air micrococci from Würzburg, which at first appeared to differ somewhat upon the plates.
10. A red micrococcus from the stomach.

The result of these comparisons was that these ten organisms all belong to the Micrococcus roseus,[1] of which we can distinguish two fairly sharply separated varieties.[2]

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[1] According to the description, the Micr. cinnabareus Flügge, cinnabarinus Zimmermann, Micr. carneus Zimmermann, may also be inserted among the varieties differentiated by us. The "new micrococcus" from red milk, recently described by Keferstein (C. B. XXI, 177), appears also very closely related. The Micr. latericius Freund (C. B. XXI, 834) appears somewhat different, yet the experiences obtained in the study of the group of the Bact. prodigiosum remind us to be cautious in the formation of new varieties.

[2] We have observed white, yellowish-red, rose-red, and carmine-red sectors upon agar in both varieties. They are connected by transition forms.

Micr. roseus (Lehm. and Neum.).

(*a*) **Typicus.**—Agar streak, rose to carmine, more rarely whitish-red. Streak upon the subtilis-potato (compare above), deep carmine-red. Milk unchanged, with beautiful rose-red precipitate. Here belong the Micr. agilis of Zimmermann from Berlin and three of our air-cocci.

(β) **Roseo-fulvus.**—Agar streak, reddish-yellow to vermilion-red. Streak upon subtilis-potato, orange-red. Milk not coagulated, with yellowish-red cream layer and yellowish-red precipitate.

Here belong, according to our investigations, Micr. tetragenus ruber Král, Micr. roseus A. Fischer, Staph. roseus Tavel, and one of our air-cocci; perhaps also the Micr. fulvus Cohn, which is very insufficiently described.

But we must go a step further still. The **Sarcina rosea** Schröter (compare p. 162) also stands in close relation to the described varieties. The Sarc. rosea, obtained from Král (it belongs to the variety roseo-fulva), forms beautiful sarcina balls upon fluid but not upon solid nutrient media, but was otherwise not to be differentiated (compare p. 163). After we had kept our ten red cocci upon hay decoction for a month, one of our red forms (from air) produced typical sarcina packets, while the others were only brought to produce tetrads.

Thus also, the Sarcina rosea may be thought of as the **forma sarcinica** of the Micrococcus roseus. The Micr. corallioides Cantani (C. B. XXIII, 309) is also very closely related, according to the description of the author, but the name "corallioides" (rectius "corallioides") is already given to another organism (p. 175).

Our point of view demands a special explanation regarding the interesting organism found by Ali-Cohen and Zimmermann in water.

Micr. agilis Ali-Cohen (C. B. vi, 33).

We have not seen *spontaneous motion* nor a *flagellum*, either in the culture from Berlin or in the one from Král, in spite of all our pains, as growing upon slant of 5% milk-sugar agar, upon sugar hay-decoction, bouillon, etc., employment of higher and lower temperatures, young and old cultures, etc. Neither culture is to be differentiated from our Micr. roseus.

Since it is not to be doubted that Ali-Cohen saw motion and Löffler, Migula, and others have stained long flagella, so we must now conceive of our Micr. agilis only as a Micr. roseus, which once possessed flagella, and then lost them.

We believe our observation is of primary significance in classification, as many investigators consider the flagella as a very important and constant differential aid. Migula has formed a genus planococcus for the Micr. agilis; without our observations we should have assented. But being in possession of this, it seems to us that our conception is at present more natural than the other possible one, namely, that the Planococcus agilis, because of the loss of its flagella, can no longer be distinguished from the Micr. roseus, but that it still belongs to a different genus.

Micr. cerasinus (List.) (Lehm. and Neum.).

Micrococcus cerasinus siccus List. (Adametz, "Bakterien der Trink- und Nutzwässer").

Very small cocci of 0.3 μ. Upon gelatin cherry-red, without liquefaction. Upon potato, dry, *spreading* deposit of cherry-red color. Pigment insoluble in alcohol and ether; whether in water, we do not know.

Micr. erythromyxa (Overbeck).

Compare Sarcina erythromyxa, page 162. Sarcina formation seems to be entirely absent at times.

Micr. cyaneus (Schröter) (Cohn).

Forms a cobalt-blue deposit, pigment soluble (!) in water, turns red with acids, blue returns with alkalies. Schröter also described a variety of this, pseudocyanea, that at first produced verdigris-green, either remaining so or later becoming bluish-green to blue. So far it has not been further described. Obtained from the air in Breslau. Regarding the **Micr. cyanogenus**, consult Pammel and Combs (C. B. L. II, 764).

II. FAMILY BACTERIACEAE (ZOPF EMEND. MIGULA).

(For diagnosis of family, see p. 124.)

1. Bacterium.[1]

Cells at least one and a half, but usually from two to six, times as long as broad, straight or bent in a plane

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[1] The "bacteria" of tuberculosis and diphtheria and those closely related to them are to be looked for in Appendix I, Actinomycetes (compare p. 127).

(compare p. 124), sometimes forming long true or apparent threads, with or without flagella. Always without endospores;[1] in single varieties arthrospores are described.

Many hundred spore-free, short rods have been described, and the need of arranging them in a natural system, founded entirely upon morphologic peculiarities, was strongly felt. The only characteristic which is questionable is that of flagella, and we acknowledge that the system founded by A. Fischer and Migula upon the flagella appealed to us very favorably until we had ourselves worked extensively with the staining of flagella. The results of these extensive and careful studies were unfortunately not of such a nature as to allow a classification founded upon the number and arrangement of the flagella to appear practical. Especially the statements in the literature regarding flagella are often inexact, and a number of inaccessible varieties could not be classified at all. At times we observed that closely related varieties, as in the colon group, occur which have either one flagellum or many or no flagella. What appeared yet worse was that, as in Bacterium violaceum, we found one form with flagella on all sides; another with only one or with one polar and one lateral flagellum. Migula found it to have one polar flagellum.

In addition, there are the experiences which we have encountered regarding the permanent loss of flagella in Micrococcus agilis Ali-Cohen, Micrococcus agilis Menge, and Sarcina mobilis, and regarding the acquiring of motility by the Bacillus implexus, and reported in this book. If we ourselves have not observed similar occurrences in any bacterium, we find the statement made by Germano and Maurea, that they have twice seen non-motile cultures of the typhoid bacterium.

Finally, we feared to scare the beginner from making differentiations if we placed before him, as the first question in the table of differentiation, the character and number of the flagella; for if the staining of flagella is no special art, yet it requires care and patience, and does not yield regularly good results even to the expert.

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[1] Upon ordinary media (bouillon, gelatin, agar, potato) these varieties never possess spores. As already remarked, we were also unable (with a doubtful exception in the Bact. violaceum) to observe spore-formation upon quince and marshmallow decoction in those previously considered as not forming spores. Migula seems to have been more fortunate, but gives no particulars.

We have therefore been required to select the appearance of the cultures in plates and the production of pigment as the important points in the separation of the bacteria, although we well know (and also always mention it) how easily the production of pigment is lost in some varieties. According to our conviction, however, at present, the proper definition of a Bact. violaceum, syncyaneum, etc., which has become colorless would constitute an (almost) insurmountable difficulty, no matter how one might construct the key for differentiation.

Key to the Recognition of the Most Important Varieties of the Genus Bacterium.

I. FORMING UPON NUTRIENT MEDIA, ROUNDISH COLONIES, WITHOUT OUTGROWTHS OR LONGER RADIATING PROCESSES, NO BRANCHES IN GELATIN STAB.

(*A*) **No growth upon ordinary nutrient media; on the contrary, very delicate growth upon inorganic saline solutions. Forms nitrate from nitrite, or nitrite from ammonia.**

Forming nitrite from ammonia, **Bact. nitrosomonas** (Win.), L. and N., page 200.

Forming nitrite from nitrate, **Bact. nitrobacter** (Win.), L. and N., page 200.

(*B*) **Scarcely any growth on ordinary media, but grows well upon pea-leaf decoction containing cane-sugar, gelatin, and asparagin. Assimilates the nitrogen of the air. Grows in the root-tubercles of leguminous plants.**

Bact. radicicola, Beijerinck, page 83.

(*C*) **Upon the ordinary nutrient media (including serum and glycerin-agar) only a very scanty growth. Delicate, drop-like colonies. Not stained by Gram's method.**

1. Small, thin, non-motile rods.
   - (*a*) For growth, the addition of a little blood is necessary. **Bact. influenzæ** (R. Pfeiffer), L. and N., page 202.
   - (*b*) Grow also without blood. **Bact. ægyptiacum** (Koch-Weeks), L. and N., page 201. **Bact. tussis convulsivæ** (Czapolewsky), L. and N., page 205.
2. Large rods arranged in pairs. **Bact. duplex** (Morax), L. and N., page 206.

3. Chains of slender rods. **Bact. ulceris cancrosi** (Kruse), L. and N., page 207.

(*D*) **Grow well upon all ordinary nutrient media, especially upon agar and gelatin.**

I. Colonies and nutrient media remain colorless.

(A) Gelatin not liquefied, organisms without flagella, non-motile.

+. No visible gas formed from grape-sugar.[1]

1. Not stained by Gram's method. When coming from the animal body they show polar staining. Form abundant acid from grape- and milk-sugar. Milk often not coagulated. Growth on potato usually poor, whitish-gray. **Bact. septicæmiæ hæmorrhagicæ**, Hüppe, page 208.[2]
2. Very similar to 1. Causes tuberculous-like changes in the animal. **Bact. pseudotuberculosis rodentium**, L. and N., page 213.
3. Very similar to 1; usually still more delicate. Tendency to formation of involution forms upon chlorid of sodium agar. **Bact. pestis** (Yersin-Kitasato), L. and N., page 213.
4. Stains by Gram's method. Grows poorly upon solid nutrient media; marked formation of acid from sugar; milk is coagulated. **Bact. Güntheri**, L. and N., page 223.
5. Stains by Gram's method. Abundant growth upon solid nutrient media. No formation of acid from milk-sugar. Milk becomes slimy. **Bact. lactis viscosum** (Adametz), L. and N., page 230.

++. Evident gas-formation from grape-sugar; closely related varieties.

1. Stains by Gram's method. Marked fermentation of milk-sugar. Milk coagulated. **Bact. acidi lactici**, Hüppe, page 220.
2. Does not stain by Gram's method.
    ×. Phosphorescence when oxygen is admitted. **Bact. phosphorescens**, B. Fischer, page 231.
    ××. No phosphorescence when oxygen is admitted (group of the Bact. pneumoniæ Friedländer).
        (*a*) Fermentation of milk-sugar with liberation of gas. Milk coagulated. **Bact. aërogenes**, Escherich, L. and N., page 221.
        (*β*) Fermentation of milk-sugar without liberation of gas. Capsules formed in animal. **Bact. pneumoniæ**, Friedl., page 225.

(B) Gelatin not liquefied. Organisms motile, with many peritrichous, rarely with one or a few polar flagella.

(*a*) No fermentation of sugar with formation of gas. Milk not coagulated. No indol formation. **Bact. typhi,**[1] **Gaffky,** Eberth, page 232.

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[1] See the remarks regarding our contradictory findings in connection with Löffler's swine plague.

[2] See also Bact. hæmorrhagicum (Kolb), L. and N.

(*β*) Fermentation of grape-sugar with formation of gas. Milk-sugar affected only slightly or not at all and without formation of gas. Milk not coagulated. **Bact. choleræ suum,** L. and N., page 252.

(*γ*) Fermentation of grape-sugar with formation of gas, milk-sugar scarcely at all affected. In growth it is between the Bact. typhi and Bact. coli. **Bact. icteroides** (Sanarelli), L. and N., page 256.

(*δ*) Fermentation of grape- and milk-sugar with formation of gas. Milk coagulated. **Bact. coli** (Escherich), L. and N., page 243.

(*C*) Gelatin not liquefied. Forms acetic acid from alcohol. More details in tables, pages 261 and 262. **Acetic acid bacteria.**

(*D*) Gelatin liquefied, or consumed without visible liquefaction. Organisms non-motile.

(*a*) Gelatin liquefied in a funnel form. Sugar fermented. Abundant growth on potato. Optimum temperature about 25°. Agar is colored reddish-brown. **Bact. disciformans** (Zimm.), L. and N., page 263.

(*β*) Gelatin consumed in a funnel form without perceptible liquefaction. No growth on potato. Optimum temperature 12°. Agar not colored. **Bact. salmonicida** (Emmerich and Weibel), L. and N., page 266.

(*E*) Gelatin not liquefied, only slowly drawn in. Spontaneously motile from polar flagellum. Stains by Gram's method. Milk unchanged. **Bact. caniculæ** (Galli-Valerio), L. and N., page 260.

(*F*) Gelatin liquefied. Organisms motile.

(*a*) Grape-sugar fermented. No branches sent out toward the solid gelatin. **Bact. punctatum** (Zimm.[2]), L. and N., page 264.

(*β*) Grape-sugar fermented. Branches sent out toward the solid gelatin. **Bact. vitulinum** (Weissenberg), L. and N., page 264.

II. Formation of a yellow (greenish-yellow to orange-yellow) pigment in the cultures of the bacteria upon agar and gelatin. (Without fluorescent discoloration of the nutrient substratum.)

(*A*) Very small, thin, short rods; upon gelatin and agar grow slowly as a thin, intensely yellowish-green layer. Gelatin very slowly liquefied. Possess a single flagellum. **Bact. turcosum** (Zimm.), L. and N., page 267.

(*B*) Short rods of the dimensions of the Bact. coli.

(*a*) Without spontaneous movement.

1. Gelatin not liquefied.

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[1] Compare Bact. typhi murium, page 258, and Bact. alcaligenes, page 257.

[2] Compare also Bact. fœtidum liquefaciens, cloacæ, agile, page 265.

(α) Culture pale grayish-orange (cream). **Bact. cremoides**, L. and N.,[1] page 267.
(β) Growth lemon-yellow. **Bact. luteum** (Fl.), L. and N., page 268.
2. Gelatin slowly liquefied.
(α) Luxuriant lemon-yellow layer on gelatin. Agar and gelatin colored red. **Bact. erythrogenes** (Grotenfelt), L. and N., page 268.
(β) Rather abundant lemon-yellow growth on gelatin. Agar and gelatin colorless. **Bact. helvolum** (Zimm.), L. and N., page 268.
(γ) Growth on gelatin at first white, then yellowish. Milk slimy. Soapy smell. **Bact. lactis saponacei**, Weigmann, page 269.
3. Gelatin rapidly liquefied. Growth upon gelatin very delicate. Little chromogenesis. **Bact. nubilum** (Frankland), L. and N., page 269.
(*b*) Spontaneous motility from polar flagellum. Gelatin liquefied, pale ocher-yellow sediment. Upon potato and agar, a pale ocher-yellow deposit. **Bact. ochraceum** (Zimm.), L. and N., page 270.
(*C*) Short rods to long threads. Cultures grayish-orange to pale orange and brick-red. Never branches in the stab.
(*a*) Non-motile. **Bact. fulvum** (Zimmermann), L. and N., page 270.
(*b*) Motile. **Bact. chrysoglœa** Zopf., page 272.

III. Formation of a rose-red to a brown-red pigment upon agar and gelatin. Especially beautiful chromogenesis upon potato. (*For red-brown and brick-red varieties, compare also Bact. fuscum and chrysoglœa.*)
(*A*) Stains by Gram's method. Non-motile. Gelatin not liquefied. **Bact. latericium** (Adametz), L. and N., page 272.
(*B*) Does not stain by Gram's method. Motile. Gelatin liquefied. Pigment rose to carmine-red, more rarely reddish-yellow. **Bact. prodigiosum** (Ehrenberg), L. and N., page 272.

IV. Formation of a non-diffusible, violet or blue pigment in the cultures upon agar, gelatin, and potato.
(*A*) Gelatin more or less rapidly liquefied. Forms a deep violet pigment, which is soluble in alcohol. **Bact. violaceum**, Schröter, page 277.
(*B*) Gelatin not liquefied. Pigment pale to deep indigo-blue, insoluble. **Bact. indigonaceum** (Claessen), L. and N., page 280.
(*C*) Gelatin slowly liquefied. Bluish-green, insoluble pigment, especially marked on potato. **Bact. cæruleum** (Voges.), L. and N., page 280.

V. The growths of the bacteria are colorless or only slightly yellowish, bluish, brownish, or greenish in color; on the contrary, a yellowish-green to

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[1] For relatives and synonyms, see the text.

bluish-green fluorescent pigment diffuses out from the culture,[1] *both in gelatin and agar.*

All varieties are provided with a single flagellum or a bunch of flagella located at the end. The group consists of varieties very closely related to each other, none of which forms gas from sugar. According to Zimmermann, all fluorescent bacteria, when young, stain by Gram's method; but according to our observations, they do not do so regularly.

- (*A*) Gelatin liquefied. Colonies in plate, after liquefaction begins, are surrounded by hairs.
  - (*a*) Intense production of pigment, usually bluish-green, upon all nutrient media, also in milk and bouillon. Milk coagulated with alkaline reaction; then coagulum is dissolved. Pathogenic for animals. **Bact. pyocyaneum** (Flügge), L. and N., page 281.
  - (*β*) Pigment production less; in bouillon very slight. Milk not coagulated; later it becomes clear and colored greenish-yellow. **Bact. fluorescens** (Flügge), L. and N., page 285.
- (*B*) Gelatin not liquefied. Colonies in plate, even-bordered, wavy, reminding one of the Bact. coli.
  - (*a*) Growth on agar and gelatin, white or yellow. No formation of blue or brown pigment aside from the fluorescent material. **Bact. putidum** (Flügge), L. and N., page 287.
  - (*β*) Besides the fluorescent pigment, there is also formed a blue, deep blue, or dark brown pigment in varying amount. Grape-sugar milk becomes blue to bluish-gray. **Bact. syncyaneum** (Ehrenb.), L. and N., page 289.

VI. The bacterial growths are pale (white to brownish colored), and through diffusion the surrounding nutrient medium is colored intensely brown.

1. Gelatin not liquefied. **Bact. brunificans**, L. and N., page 292.
2. Gelatin liquefied. **Bact. ferrugineum** (Rullmann), L. and N., page 292.

II. COLONIES UPON THE NUTRIENT MEDIA ARE ROUNDISH AT THE BEGINNING ONLY, IF AT ALL; LATER, THERE EXTEND MORE OR LESS FROM WITHIN OUTWARD, RAY-, FORK-, BAND-, OR SAUSAGE-LIKE OUTGROWTHS.

In the Bact. vulgare, where these outgrowths may be absent, one observes—best in 5%-6% gelatin—a swarming in the periphery of the colonies in the plate. In the gelatin stab culture there sometimes occurs the formation of branches. (Genus: **Proteus Hauser.**)

(*a*) With spontaneous motion and peritrichous flagella.

1. Gelatin not liquefied. Branching very beautifully developed. Causes putrid decomposition. **Bact. Zopfii** (Kurth.), L. and N., page 293.
2. Gelatin usually liquefied. No branching. Causes intensely

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[1] For transition forms between these varieties, consult the detailed descriptions.

putrid decomposition. **Bact. vulgare** (Hauser), L. and N., page 295.

(*b*) Without spontaneous motion and flagella. Gelatin slowly liquefied.

1. Gelatin colony resembles a bone corpuscle; delicate center, with a series of irregular outgrowths. In gelatin stab: nodules, prickly balls, and branches. **Bact. erysipelatos suum** (Löffler, Schütz), Migula, page 302.
2. Gelatin plate similar to the above, or (usually) with very delicate, almost invisible colonies. Branches in the stab culture are very delicate and regular. **Bact. murisepticum** (Flügge), Migula, page 300.

Bacterium nitrosomonas (Winogradsky), Lehm. and Neum.[1]

Nitrosomonas europæa (Winogradsky). A. P. IV, V; and Arch. des sciences biolog. de Petersbourg, I, 1892. The morphology is very briefly described. Elliptical and short spindle-shaped, quiet cells, often united in short chains (about 1 μ broad, and 1.1–1.8 μ long). Upon silicic acid nutrient media the organisms form compact, sharply contoured, brown colonies, from which, after about two weeks, motile swarms wander out (appearing as a pale halo). In fluids there is first a slight sediment; then after about eight days diffuse cloudiness due to the motile form, which in one or two days again settles quietly to the bottom.

The organisms thrive only upon inorganic nutrient media: gelatinous silica or water to which is added, in a liter, about 1.0 gm. potassium phosphate, 0.5 gm. magnesium sulphate, a trace of chlorid of calcium, 2.5 gm. ammonium sulphate, and some solid magnesium carbonate. They form nitrite, but no nitrate, from salts of ammonia.

Growth of the pure culture is difficult, and so far but rarely accomplished.

Bacterium nitrobacter (Winogradsky). L. and N.

Literature.—Winogradsky (C. B. L. II, 415); Winogradsky and Omeliansky (C. B. L. V, 329). The statements of Burri and Stutzer, as also those of Stutzer and Hartleb, regarding a polymorphous saltpeter fungus are incorrect. Compare Fränkel (C. B. L. IV, 8, 62) and Gärtner (C. B. L. IV, 1, 52, 109).

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[1] We select this name because it has many advantages over the unning one of Bact. europæum.

Microscopic Appearance.—Short rods, 1 μ long, 0.3–0.4 μ thick. Stain poorly. When stained with warm gentian-violet solution and washed with a 10% solution of chlorid of sodium, a stained capsule surrounds the bacilli, which are unstained. With carbol-fuchsin the rods are gradually stained, the pointed ends escaping. Alkaline methylene-blue first stains the ends, then the central portion.

Motility is never observed. No growth occurs upon the ordinary nutrient media, rich in organic substances (bouillon, agar, gelatin), but it grows upon the following: Nitrite-agar, which contains pure sodium nitrite 2 gm., sodium bicarbonate 1 gm., potassium phosphate and agar 15 gm., water 1 liter; or nitrite solution, which contains: sodium nitrate 1.0, potassium phosphate 0.5, magnesium sulphate 0.3, sodium bicarbonate 0.5–1.0, sodium chlorid 0.5, a little iron sulphate, distilled water (distilled twice over permanganate) 1000. If soda is used instead of sodium bicarbonate, then also free CO_2 must be present. The addition of more than 0.4% peptone, or of small quantities of sugar, prevents the growth and the production of nitrate.

Nitrite-agar Colonies.—*Deep:* granular, dense, small, sharply outlined, strongly refracting, appearing only after weeks. On the surface delicate, cloud-like, homogeneous, scarcely at all granular droplets develop equally slowly.

Nitrite-agar Stab Culture.—Somewhat more luxuriant, dirty white, greasy.

Isolation from Soil.—Numerous plates are prepared from nitrite-agar with larger and smaller quantities of soil suspended in it. After standing for three or four weeks at about 20°, test the plates to determine whether nitrate has been formed. Inoculate from a number of the smallest colonies into nitrite solution, and after about three weeks prepare new plates of nitrite-agar from the tubes which contain no nitrite, but nitrate. The pure culture should behave as follows: (1) A scarcely perceptible precipitate should appear, which rises as a column on shaking; (2)

upon gelatin and agar plates no colonies develop; (3) in tubes with nitrite solution the reaction for nitrite should disappear after about eight days.

Bacterium influenzæ (R. Pfeiffer). Lehm. and Neum.

Literature.—R. Pfeiffer (Z. f. H. XII, 357, 1893), with 7 plates; Delius and Kolle (Z. H. XXIV, 327) (immunity, production of toxins); Grassberger (C. B. XXIII, 25).

Microscopic Appearance.—Very small, short rods, about 0.4 μ broad, 1.2 μ long, often in pairs, often in sputum within the cells, more rarely united in short threads (68, V). Grassberger observed typical cultures with a marked tendency to form thin and thicker apparent threads,[1] which in part were swollen into spindle-form, and at times branching could be seen. This must be studied further.

Spontaneous motion is absent.

Staining Properties.—Somewhat poorly with the ordinary aqueous solutions of anilin dyes, better with alkaline methylene-blue, and best by the application of a very dilute carbol-fuchsin solution for five minutes. With faint staining, the ends are somewhat more deeply stained. Not stained by Gram's method.

Relation to Oxygen.—Obligate aerobe.

Requirements as Regards Nutrient Media and Temperature.—Grows only upon agar smeared with blood (or hemoglobin) or blood-bouillon. Optimum, 37°. Upper limit, 43°; lower, 26°–27°.

Agar Streak.—(Surface smeared with blood.) Clear, like glass, small, hardly confluent, almost structureless colonies.

Bouillon with Addition of Blood.—If the nutrient medium is placed in a thin layer, the Bact. influenzæ develops as delicate, white flocculi.

Special Nutrient Media.—According to Grassberger, a mixture of agar and defibrinated blood, heated for one hour to 50°–60°, is a specially favorable nutrient medium. According to Grassberger, the influenza bacterium grew with very much greater luxuriance upon unheated blood-media in proximity to colonies of the Micr. pyogenes. It may be supposed that heat and the growth of the Micr. pyogenes alter the blood-medium in a similar manner (Z. H. XXV, 453).

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[1] The pseudo-influenza bacilli described by R. Pfeiffer (*l. c.*) grow as large thick rods and false threads, but are identical with the I. B., according to Grassberger.

Vitality and Duration of Life.—In water, even in the dark, they die in from twenty-eight to thirty-two hours; in agar and bouillon cultures, after two or three weeks. In fresh sputum they are preserved about the same length of time. Rapid drying kills in two hours; slower drying, in from eight to twenty-four hours.

Distribution.—(*a*) *Outside the body:* Not found.

(*b*) *In influenza in man:* Very abundant in the characteristic, clear, yellowish-green, lumpy, tenacious sputum. They are found purest in the secretion of the finer bronchi; at first free in clumps, later especially within the pus cells. Also, extensive colonization occurs in the lung tissue, leading to lobular and pseudo-lobular influenza pneumonia. They are often abundant in the nasal secretion in cases of influenza. R. Pfeiffer found them rarely in the blood, and never cultivated them from the blood. In the organs, especially the brain, they are demonstrable relatively seldom (Nauwerck, C. B. XVIII, 395; Pfuhl, Z. H. XXVI, 112). E. Fränkel traced a suppurative meningitis to the I. B. alone (Z. H. XXVII, 315).

Animal Experiments.—Influenza can be transferred to the monkey only, among all the numerous available experimental animals. Devitalized cultures in large quantities are intensely toxic (dyspnea, paralysis) for animals, especially rabbits.

Immunity and Serum Reaction.—Animals which are treated for a long time with I.-toxins do not yield a serum with antitoxic or bactericidal properties, but succumb to infection with a larger quantity of culture (Delius and Kolle).

Special Culture Methods.—The bronchial mucus washed in sterile water is triturated somewhat superficially with a little sterile water; and of this, small quantities are smeared over slanted agar and slanted agar smeared with blood. If the first remain sterile, while delicate, drop-like colonies develop upon the second, it speaks in favor of influenza. Bouillon and agar mixed with sterile pigeon blood are highly recommended.

Related Varieties.—The "**bacillus of pneumonia in rabbits,**" cultivated by Beck, R. Pfeiffer's assistant, from rabbits dying spontaneously, is closely related (Beck, Z. H. xv, 363, 1893). Small, fine, non-motile bacilli, twice as long and thick as the influenza bacillus, obligate aerobe, not stained by Gram's method. Does not grow on potato. Upon gelatin it resembles the streptococcus. Upon agar, grayish-yellow, with granular, sharp border, of tough, mucoid consistency. Guinea-pigs, rabbits, and mice are susceptible. Principal changes upon section are pulmonary hyperemia and atelectasis, and fibrinous deposit upon the pleura.

Bacterium ægyptiacum (L. and N.).

Ordinary Name.—Koch-Weeks' bacillus.

Entire literature by Kamen (C. B. xxv, 449), with beautiful photographs.

Microscopically, very small, thin rods (1–2 μ long); in recent cases are often exceedingly numerous in the secretion from the eye; sometimes they form short chains. Non-motile, do not stain by Gram's method. The cultures resemble in every way those of the influenza bacillus; their growth is always poor, best upon nutrient media smeared with blood. Optimum at 37°. They live only a short time—about four days. Scarcely at all pathogenic

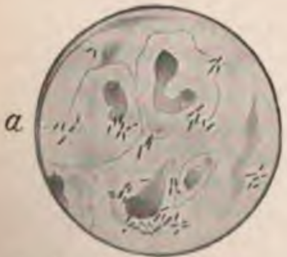

Fig. 16.—Bact. ægyptiacum (L. and N.).

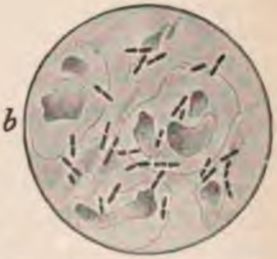

Fig. 17.—Bact. duplex (L. and N.).

for animals. Often associated with organisms of the xerosis group.

A differential diagnosis from the Bact. influenzæ appears at present scarcely possible.

It produces in Europe, especially in summer, epidemic conjunctivitis. The disease develops gradually during two or three days; after three or four days the inflammation is more severe, and may be accompanied by abundant purulent secretion. The affection continues severely for a week, and more lightly for two or three weeks.

Frequent in Egypt (Koch), but also observed in England, Paris, Hamburg, Czernowitz, as the cause of epidemics. Never has been observed in Würzburg.

Bacterium tussis convulsivæ (Czaplewski and Hensel), L. and N.

Literature.—Czaplewski and Hensel (Deut. med. Woch., 1897, 586, and C. B. XXII, 641); Koplik (C. B. XXII, 222) and Czaplewski (C. B. XXIV, 865); Zusch (Münch. med. Woch., 1898, 712, and C. B. XXIV, 721 and 769); Vincenzi (C. B. XXIV, 850). (See also Koplik, Johns Hopkins Hospital Bulletin, IX, 79, 1898.—ED.)

Microscopic Appearance.—In smears of the expectorated mucus small short bacilli, often only oval forms, of 0.75 to 1.5 μ in length. Sometimes united in very short chains (68, I). Koplik describes individuals in old cultures with slightly clubbed ends. In glycerin and sugar-agar there are sometimes longer forms, reminding one of the coryne-bacteria.

Spontaneous motion absent (according to Koplik, present).

Staining Properties. — Tendency to polar staining when dilute staining solutions are employed. Strong staining solutions give the organisms a plumper appearance.

Relation to Oxygen.—Facultative anaerobe.

Intensity of Growth.—Usually very modest; often an inoculation from a culture one day old upon the original plate is without result. Growth on agar, poor; better on glycerin-agar; best upon Löffler's serum.

Temperature.—Not below 25°; grows well only in the incubator.

Spores are absent.

Serum-agar Plate.—Young colonies exceedingly delicate, like dewdrops; single colonies may be as large as 2 mm., grayish, somewhat lobulated. Rarely are they elevated like a pinhead. Magnified from sixty to a hundred times they are finely granular.

Agar Plate.—Smaller, poorer, and usually drier than upon serum. On the contrary, Vincenzi observed better growth on agar than on serum. He describes, in the agar stab, faint growth in the stab canal and no surface growth.

Bouillon Culture.—Scanty growth, little turbidity.

Milk and potato growths are so far unknown.

Resistance to drying is minimal.

Distribution.—According to the authors previously mentioned, the organism is regularly present in the glairy, transparent sputum of typical cases of whooping-cough, and from it it has often been cultivated. We have also made, with Dr. Hirai, a series of successful cultures. We always found the cultures to remain alive for an extraordinarily short time.

Animal experiments have failed with all animals and in the hands of all investigators.

Special Cultures and Methods of Recognition.—The sputum is obtained with as little contamination as possible (when possible, after washing out the mouth), and typical, glairy, tenacious balls are allowed to stand in abundant sterile water about one hour. The water is then poured off, and the clumps washed in several changes of distilled water, and finally from them smears on cover-glasses and streak cultures upon ascites-agar are prepared.

Bacterium duplex (L. and N.).

Ordinary Name.—Diplobacillus Morax.

Literature.—Morax (A. P. x, 337); Axenfeld (C. B. XXI, 1), with plate.

Microscopic.—Rather large, plump rods, often arranged in pairs or short chains, about 1 μ thick and 2 to 3 μ long, non-motile, not stained by Gram's method, without any capsule of importance.

The organisms are very particular as to cultivation. They grow best upon ascites-agar as small transparent droplets; upon ordinary agar, a growth is rarely obtained. Pure, solidified blood-serum is slowly liquefied on the surface. Cultures have little durability. It causes a conjunctivitis, usually insidious in onset and running a chronic course with slight catarrhal symptoms, abundant secretion, and redness of the conjunctiva, especially upon the edges of the lids and inner angle of the eye. The organism is found abundantly in the secretion (Fig. 17). The disease may be transferred by means of pure cultures to healthy individuals. It has been found infrequently in various places as the cause of epidemics; also, on one occasion, in Würzburg.

Bacterium ulceris cancrosi (Ducrey-Kruse), L. and N.

Synonyms.—Streptobacillus of soft-chancre Ducrey, Bacillus ulceris cancrosi Kruse.

Literature.—Ducrey (C. B. XVIII, 290), Petersen (C. B. XIII, 743), Unna (C. B. XVIII, 234), Kruse (Flügge-Kruse, Bd. II, 456).

It is now universally acknowledged that Ducrey rightly recognized a small, thin bacterium (0.5 μ broad, 1.5 μ long), arranged in long chains, which can be demonstrated, with no great difficulty, in sections of soft chancre as the cause of the process. By successive inoculation of chancre secretion from one place on the skin to others, in each resulting ulcer a purer condition is found. Staining of the sections with Löffler's methylene-blue is not especially difficult, if the alcohol is allowed to act very briefly. The bacteria are not stained by Gram's method. They are also found in the chancre secretion, but only rarely in the contents of buboes. Cultures are rarely successful; Petersen obtained non-characteristic, faintly growing colonies deep in serum-agar.

Bacterium septicæmiæ hæmorrhagicæ.[1] Hüppe.

(Plate 12.)

Literature.—Complete by Voges (Z. H. XXIII, 261; XXVIII, 33); Karlinski (Z. H. XXVIII, 407); Th. Smith (C. B. XXV, 241); Voges and Proskauer (Z. H. XXVIII, 20); Preisz (C. B. XXIII, 666).

Microscopic Appearance.—Short rods, from the animal scarcely ever more than twice as long as thick, very small (0.3–1 μ long). Very often (always typically) in the short rods with somewhat smaller ends only the poles stain (plasmolysis) (12, IX and schematic, 12, X), so that pictures resembling diplococci result. Heim once observed typical capsules. In cultures, likewise, there are mostly short rods (12, IX), rarely short threads.

Spontaneous motility and **flagella** are absent.

Staining Properties.—Not by Gram's method.

Dependence upon Temperature and Nutrient Media.—About like the Bact. coli. Facultative anaerobe.

Growth upon Agar and Gelatin.—As shown in Plate 12, differing but little from the Bact. coli.

Milk Culture.—Behave differently. Our Berlin chicken cholera presents the typical properties; it renders milk alkaline and leaves it fluid; similar effects are produced by a culture of Löffler's swine plague from Berlin and one Honl. On the contrary, one obtained from C. Fränkel gulated milk with formation of acid.

Potato Culture.—Often no growth, especially when ltivated freshly from the animal, or only a very scanty one. Old laboratory cultures grow as faintly yellowish-white; after alkalinization the growth is more abundant.

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[1] Our description is based upon a culture of "chicken cholera," tained from the Hygienic Institute in Berlin, whose properties agree xcellently with those described in the literature. Two cultures, of "chicken cholera" and "rabbit septicemia," which have been cultivated in our institute for about six years, and which originally came from a trustworthy but now unknown source, behave like typical Bact. coli in the sense of the definition in our key. Unfortunately, the connection between these can not be explained; a contamination seems to be excluded, a transformation is improbable. One might see a proof in this observation of the identity of the Bact. sept. hæmorrhag. with the motile organism of hog-cholera, so long maintained especially by Voges. It does not seem possible to draw more far-reaching conclusions from the observation, especially as Voges and Proskauer now again maintain sharp differences between the causes of the diseases from the biologic characteristics (fermentation of sugar,

Production of Gas and Acid from Carbohydrates.—Often much acid is formed, both from grape- and milk-sugar, but no gas.[1]

Indol and H_2S.—Both abundantly formed (according to Karlinski, not). According to Hoffa, methylguanidin is to be looked upon as the poisonous principle of the organism.

Resistance.—Against drying, slight. Heating to 45°–46° destroys the virulence in half an hour. On the contrary, cultures remain viable and virulent for months. Cold and mixture with putrefactive bacteria do not reduce the virulence.

Distribution.—(*a*) *Outside the body:* Demonstrated by Gaffky in water of the Panke. Inoculation of the same into rabbits produced a fatal infectious disease. Also found in water and soil; undoubtedly widely distributed.

(*b*) *In the body:* Never in man. On the contrary, they were found by Gamaleia in the feces of normal pigeons, but with little virulence, and by Karlinski in the nasal mucus of swine. Have been demonstrated to be the cause of a series of destructive diseases in animals, in various biologic races, and designated by various names.

Voges was unable to produce, in any way, a true, lasting immunity against any of those diseases.

We will describe only four of these varieties somewhat more extensively.

1. Bacterium suicida Migula (Bacillus suisepticus Kruse), cause of the so-called German (Löffler's) "Schweineseuche." Compare Löffler and Schulz (A. G. A. I, 55 and 376). It is a wide-spread and destructive disease of swine, which usually kills in from one-half to

etc.) recently in use. We remain, therefore, for the present with a preponderating majority of authors who occupy the standpoint of a duality. A new culture, obtained from Honl, in Prague, of Bact. suicida Mig. corresponds with the scheme.

[1] This statement in the literature, which Th. Smith recently again verified, corresponds to our cultures of chicken cholera and new "Schweineseuche"; on the contrary, both of the old motile cultures of "Schweineseuche" produce gas from grape-sugar. According to Karlinski, sometimes there is formation of gas, and sometimes none.

two days. Usually a lobular, multiple, necrotic pneumonia is most prominent. Many cases pass as croupous pneumonia; other forms, with bacteria of less virulence, lead, during a chronic course, to the formation of multiple caseous areas, which are often confused with tuberculous areas. Compare Ascher and Hirsemann (Z. H. XXVI, 143). Also diseases of the intestine (gastro-enteritis) occur when a complication or secondary infection by the Bact. choleræ suum is not present. Swine are very susceptible, and, of the experimental animals, guinea-pigs; birds are very slightly affected.

For an exhaustive differential diagnosis from American "Schweineseuche," see page 239.

2. **Bacterium multocidum**[1] (Kitt), L. and N. (Bact. bipolare multocidum Kitt, Bac. bovisepticus Kruse), cause of the "Wild-" and "Rinderseuche" (Bollinger, Kitt), which, while not very frequent, still has raged very destructively among deer and cattle.[2] Hogs are rarely affected.

There are found hemorrhagic enteritis, with either pleuropneumonia and pericarditis, or very acute edema of the head and neck, with hemorrhages in the mucous membranes of the head.

3. **Bacterium of Barbone in buffalo disease** in Italy and Hungary (Oreste and Armanni, 1886; von Rátz, C. B. XX, 289; Sanfelice, Loi and Malato, C. B. XXII, 32). Buffalo die in from twelve to twenty-four hours; there is severe hemorrhagic edema of the subcutaneous connective tissue, especially about the larynx, trachea, etc., with the small intestine reddened and hemorrhagic. It is pathogenic for guinea-pigs.

4. **Bacterium avicidum** Kitt, **cuniculicida** (Gaffky) Flügge (Bacillus choleræ gallinarum Kruse). It is the cause of extensive epidemics in chickens (chicken cholera, Perconcito, Pasteur), isolated by Gaffky from canal-water (Mitt. Gesundheitsamt I, 80) and described as the cause

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[1] Closely related: "New Infectious Disease of Cattle," of Basso (C. B. XXII, 537). Stained by Gram's method, non-motile, ferments glucose.

[2] According to Gmelin, the causes of many cases of infectious inflammation of the navel also belong here (C. B. XXIII, 295).

of rabbit septicemia (Davaine's septicemia). It is differentiated from Nos. 1–3 by a more abundant growth on potato, and in milk sufficient acid is formed to produce coagulation.

For chicken cholera the following are susceptible: Chickens, turkeys, ducks, geese, pigeons, in general all domestic fowls, sparrows, finches, rabbits, and white mice. Usually guinea-pigs are slightly susceptible. Compare, on the contrary, Tjaden (C. B. XXV, 224). Every method of inoculation (also with only very small quantities) as well as feeding are successful, death occurring in birds usually after from twelve to forty-eight hours, rarely after from seven to twelve days. Superficial inoculation by cuts into the pectoral muscles with a lancet is most generally employed.

Postmortem Findings.—In pigeons, at the place of inoculation in the muscle, there is a whitish-yellow, thick, nodular swelling and discoloration of the muscle; in hens often more of a cloudy, edematous infiltration—an appearance of diagnostic value. Dead animals have large ecchymoses in the serous membranes (especially in the pericardium), besides serous or fibrinous pericarditis, hemorrhagic enteritis, and serous lobular pneumonia (Kitt). (Dogs and cats devour dead birds without injury.) During life the birds present suddenly developing choleraic symptoms, together with loss of appetite, weakness, giddiness, ruffled plumage, thirst. Rabbits and mice die quickly, without local manifestations, or an abscess forms at the point of inoculation, which for weeks contains the characteristic bacteria.

Special Methods of Demonstration.—Inoculation of a pigeon by very shallow cutaneous incisions in the breast, 2–3 cm. long. Characteristic organisms abundantly present in the blood of the inoculated animal (bipolar staining); change at the point of inoculation (necrosis).

The bacillus gallinarum E. Klein is a variety (C. B. V, 689; VI, 257; and XVIII, 105).

Closely related are: The disease of ring-doves of Leclainche (A. P., 1894, 490) and the duck cholera of Cornil and Toupet (C. B. IV, 333); hens are immune to both these latter. Similar also are the parrot cholera (Nocard), Fiorentini's septicemia of swans (C. B. XIX, 932), and a series of diseases in animals, which have usually been observed but once.

Bacterium hæmorrhagicum (Kolb), Lehm. and Neum.

(Plate 20, VII, VIII.)

Literature by Babès (C. B. IX, 719); Kolb (A. G. VII, 60); Afanasieff (C. B. XIII, 402); Finkelstein (C. B. XVIII, 64).

Very closely related to, indeed, only biologically different from, the Bact. septic. hæmorrhag. is an organism closely studied by Babès, Tizzoni, and Giovannini, but especially by Kolb (illustration, literature), which causes purpura—Morbus maculosus Werlhofii—in man and experimental animals, which usually terminates fatally. There occur hemorrhages into the skin, serous membranes, lungs, kidneys, etc., and albuminuria.

Microscopic Appearance.—Short, oval bacteria, 0.8–1.5 μ long, 0.4–0.8 μ thick, usually in pairs (20, VII) with a small capsule in the animal body; in cultures, short rods and threads. Non-motile. By Gram's method they stain poorly or not at all. Facultative anaerobe.

Gelatin Culture.—Grow rather slowly; delicate, thin, whitish, spreading but little, never liquefying. **Agar culture:** Uncharacteristic, white to whitish-yellow, spreading somewhat flatly. Upon potato, whitish, moistly glistening, not spreading much, not tenacious. Regarding the relation to sugar solution nothing is stated; since in anaerobic cultures, which bear the addition of sugar well, nothing is said by Kolb of gas-formation, it does not appear to cause fermentation. The varieties isolated by the three above-mentioned authors were different in their pathogenic effects upon experimental animals. Kolb obtained the greatest effects upon mice, less in guinea-pigs and dogs; the organism of Tizzoni and Giovannini, on the contrary, was not pathogenic for mice, but very pathogenic for dogs and guinea-pigs. The animals often present marked hemorrhages, with the same localization as in man.

Bacterium pseudotuberculosis rodentium. Preiss. (L. and N.)

Synonym.—Bacillus pseudotuberculosis A. Pfeiffer.
Entire Literature.—Delbanco (Ziegler's Beiträge XX, 477).

Microscopic.—Plump, short rods, motility absent or doubtful, flagella not found, often arranged in short chains in cultures, stain best with alkaline methylene-blue, not by Gram's method.

Cultures.—Somewhat like Bact. coli, grow readily and luxuriantly upon most nutrient media (only upon potato poorly), forming yellowish-white to salmon-colored and yellowish-brown growths. Bouillon is first diffusely cloudy, then presents a thick sediment, but no pellicle. Abundant formation of crystals in the cultures from the formation of alkali (basic phosphate). No fermentation of sugar with formation of gas. Milk is not coagulated. No indol.

Distribution.—Frequently found as the cause of tuberculous-like, caseous, granulation swellings (especially in the abdomen) of rodents (rabbits, guinea-pigs). Appears widely distributed; may also cause epidemics.

Detection.—The organism may be easily found in stained smears from the swellings. Growth occurs readily, and thus it is differentiated from tuberculosis.

Bacterium pestis.[1] (Kitasato, Yersin.) L. and N.

(Plate 13.)

Literature.—Yersin (A. P. VIII, 662); Aoyama (C. B. XIX, 481); Ogata contra Kitasato (C. B. XXI, 771). Much newer literature is cited in the text. Especially important is: Gaffky, R. Pfeiffer, Dieudonné, Sticker. Report of the German Pest Commission (A. G. A. XVI, 1899).

Microscopic Appearance.—Short rods with rounded ends, two to three times as long as thick, here and there united in pairs (13, X *a*). In smear preparations from exudates or fresh portions of the body, the organisms show the usual polar staining with anilin dyes, as in those of septicæmia hæmorrhag. (13, IX). In bouillon there occur streptococcus-like chains (13, x *b*). The bacteria are provided with a capsule, but it is not easily rendered visible (Report of the German Pest Commission). In connection with bacteria from a pure culture, we have not often seen them, yet they may at times be demonstrated by using dilute staining solutions.

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[1] We have had at our disposal for study and illustrating, through the great kindness of Dr. Dieudonné, besides the living cultures, also a series of preserved original Indian cultures and original preparations.

Spontaneous Motility.—Absolutely non-motile, no flagella. It must be noted that Kitasato observed very sluggish motion, and likewise Kasanski saw movement of the bacteria (C. B. XXIII, 25). Gordon stained, according to the method of van Ermengen, flagella, which are usually single and polar, rarely in pairs and at the sides (C. B. XXII, 170); compare also N. Schultz (C. B. XXIII, 594). According to the statement of the German Pest Commission, what was supposed to be motility was only molecular motion, and the flagella observed may be supposed to be simply precipitated staining materials.

Staining Properties.—Stain with all anilin dyes. In preparations from pure cultures, the polar staining is not clearly observed. Not stained by Gram's method. In opposition to Kitasato, the statement is made that the bacteria in the blood stain by Gram's method. According to Kasanski, the polar staining succeeds especially in blood and old pus.

Relation to Oxygen.—The bacteria are obligate aerobes. Growth is stopped by the exclusion of oxygen.

Dependence upon Temperature.—Optimum 37°, but it also grows very well at 22°.

Intensity of Growth.—On all nutrient media, tolerably rapid. After two or three days a luxuriant deposit is observed. In bouillon diluted with three times its quantity of water, the growth is very much slower. In dilutions of 1 : 10 it is almost entirely absent (Report of the German Pest Commission).

Liquefaction.—Absent.

Spores.—None formed. The vegetative cells die completely at 55°–60°.

Involution Forms.—Very characteristic and remarkable involution forms are produced, the like of which are said to occur in no other variety. The cell bodies are swollen in the center, similar to yeast cells, or they become rounded, like spherical forms. Very often there appear cells many times larger than normal cells. The staining properties in these forms are lessened (13, VIII). According to the statement of the German Pest Commission, upon Hankin's 3% chlorid of sodium-agar involution forms are produced almost exclusively (C. B. XXII, 438).

Gelatin Plate.—(*a*) *Natural size:* Small, crumbly, gray, transparent colonies, which are directly elevated above the surface. After a longer time, they spread out but little (13, V *b*).

(*b*) *Magnified sixty times:* Answering to the elevation which occurs, there is observed a marked reflection from the surface. The colonies are roundish, smooth-bordered to lobulated, sharply outlined, with a yellowish to a greenish shimmer. Very often the superficial colony is surrounded by a very delicate, transparent lobulated zone, which occurs also in somewhat altered form upon other nutrient media. The structure varies from homogeneous to faintly granular. The deep-lying colonies are similar, but never present this delicate zone (13, VI).

Gelatin Stab.—In stab-canal, a faint, homogeneous, whitish, thread-like growth. On the surface the growth is like the colony in the gelatin plate.

Agar Plate.—The description refers to cultures which have been cultivated a long time. Cultures recently obtained from pest cadavers behave somewhat differently. (See under agar streak.)

(*a*) *Natural size:* After forty-eight hours the colonies are plainly visible macroscopically, have wavy, smooth borders, are slightly elevated, and cannot be differentiated from those of the colon bacterium. They are gray to grayish-white, and have an oily or moist luster (13, V *a*).

(*b*) *Magnified sixty times:* Colonies roundish, transparent at the periphery, in the interior yellowish to yellowish-gray. They are universally very crumbly. Sometimes one is reminded of a very granular colony of diphtheria or of a delicate sarcina colony (13, VII *a*). The better the nutrient medium, the more luxuriant the growth. Thus the colonies on glycerin-agar (13, VII *b*) and ascites-agar (13, VII *c*) are less transparent and darker in color.

The delicate, tiny, drop-like colonies which occur when cultures are made direct from pest material (13, II), when magnified sixty times, appear less crumbly, sometimes homogeneous, almost smooth-bordered, and elevated like the head of a pin. Here, as in the colonies in gelatin, the older colonies present a very delicate, transparent, finely punctated peripheral zone, upon which the colony proper appears built up as a hemisphere (13, VI). Young colonies are gray; old ones, grayish-yellow to brownish-gray.

Agar Stab.—Difficult to differentiate from the colon bacterium. The surface growth is somewhat whiter than with the colon. Stab is uncharacteristic and thread-like.

Agar Streak.—The growth upon the surface from fresh pest material consists of tiny, delicate, transparent, drop-like colonies, which lie close to each other and appear as a delicate surface-layer. The minute colonies are not confluent and do not become much larger. The entire layer appears grayish-yellow (13, II). The growth of cultures which have been long under cultivation cannot be differentiated from that of a well-developed colon culture, but is perhaps a little whiter.

Bouillon Culture.—At first is slightly turbid; in course of time a pellicle forms, which at first is delicate, and later becomes denser. Very old cultures are often clear, with an abundant, crumbly sediment. In sugar bouillon the sediment is more marked, also the pellicle is more luxuriant.

Milk Culture.—Growth in milk is slight; the milk is not coagulated.

Potato Culture.—Grows slowly. Deposit is whitish to whitish-yellow, faintly shining, somewhat elevated, crumbly. It is sharply outlined from the potato.

Special Nutrient Media.—Upon boiled rice, at 30°–37°, there is an abundant growth in the form of a gray film (Report of the German Pest Commission).

Chemical Activities.—(*a*) Chromogenesis, production of odoriferous and gustable substances, liquefaction of gelatin, and H_2S are absent.

(*b*) *Indol reaction:* After a long time; without the addition of nitrite, slight; with the addition of nitrite, pronounced.

(*c*) *Toxins:* Fluid cultures, devitalized by heat, never contain soluble toxin. By extraction from cultures eight to twelve weeks old which are killed with formalin, a fluid is obtained which is very rich in toxin, and from this, with ammonium sulphate or alcohol, a solid toxin may be obtained, of which $\frac{1}{72000}$ of the body-weight is fatal for mice. Still, in the serum of animals treated with large doses of toxin antitoxins are absent (Wernicke, C. B. XXIV, 859). Markl obtained similar results. He obtained the largest quantities of toxin in shallow bouillon cultures quite rapidly (in a few days); he obtained sera with limited antitoxic action, but without any effect against infection with living bacteria (C. B. XXIV, 641). Roux, who produced strongly active sera, found them strongly antitoxic, but not bactericidal.

Resistance and Viability.—The pest bacillus is not very different from other fission-fungi. It withstands drying from three to seven days; in water it dies in from three to eight days, according to the composition. In buried bodies the duration of life is from twenty-two to thirty-eight days. Kasansky proved that they stand the Russian winter for months (C. B. XXV, 122). For particulars consult Toptschieff (C. B. XXIII, 730), Gladin (C. B. XXIV, 588), Hankin (C. B. XXIV, 587), Wladimiroff (C. B. XXIV, 424).

Distribution.—(*a*) *Outside the body:* In India, Hankin and Yersin have many times cultivated non-virulent varieties of bacilli, resembling very much the pest bacillus, from the environs of men in houses infected with pest.

(*b*) *In healthy body:* Never.

(*c*) *In diseased human body:* Is widely distributed. Most abundant in the buboes, primary cutaneous pustules, and the sputum of pest pneumonia. Rarely found in the blood and organs (compare below).

(*d*) *In animals:* Pest occurs spontaneously in rats. Epidemics of pest in rats often precede those in man. It appears as if certain tropical soil bacilli first become acclimated to the rat's body, and then are transferred to man.

Pathogenic Properties for Man.—Cause of the true oriental bubonic or glandular pest; also of the pest pneumonia. Mortality 50%–80%. The gates of entrance are: (1) Skin. The bacteria usually first become localized and grow in the nearest lymph-glands (gland-pest), but often there develops at the place where the bacterium enters a pest pustule, which may be of the character of a boil or carbuncle, and may contain very many bacteria. Death may occur without the pest bacteria extending beyond the local area, but usually it follows a dissemination of the bacteria throughout the entire body (pest sepsis). Rarely pest bacteria also occur in great numbers in the internal organs; at times in the urine. (2) Lungs; pest pneumonia. In the sputum there are very numerous pest bacteria, often also in the blood. Complication with streptococci is frequent. (3) Digestive canal; uncertain. In animals it has been demonstrated.

Experimental Investigations Regarding Pathogenic Effects.—Almost all animals are susceptible to pest. Pigeons are immune; dogs and cows but slightly susceptible (Gosio, H. R., 1897, 855); more susceptible are swine, horses, cats; yet more, monkeys and rabbits; and most of all, guinea-pigs, mice, and rats. Compare Nuttall (C. B. xxii, 87). The pest bacillus may also become acclimated to frogs (Devell, C. B. xxii, p. 382).

Guinea-pigs inoculated intraperitoneally die in two days of an acute septicemia with few bacteria in the tissues. After infection with small quantities of pest bacilli, death occurs on the sixth day, when the mesenteric glands are swollen and there are hemorrhages in the liver and lungs, together with submiliary abscesses and nodular thickenings of the omentum. The spleen contains whole swarms of bacteria, which are united in a zooglear mass. These zoogleæ are formed by very much swollen capsules. Honl (C. B. xxii, 100).

Guinea-pigs are easily infected through the digestive tract, in which case there is a special tendency to chronic forms. (Nodules in various organs, including lungs.) Bandi and Stagnitta-Balistreri (Z. H. xxviii, 261).

Flies transport pest bacilli; bugs and fleas remove pest bacilli together with the blood from animals with pest, yet a transfer to healthy animals appears rare.

Immunity and immunization (consult the report of the German Pest Commission and Dieudonné, Münch. med. Wochenschr., 1898, 166).

Passive immunity may be obtained in animals, and to a certain degree also in man, by the subcutaneous injection of serum from horses which have previously been treated many times with intravenous injections of devitalized cultures; curative power is also possessed by such serum over sick men and animals, yet only in a modest measure and in very large doses. According to Roux, the action of the serum is only antitoxic, not bactericidal. According to Haffkine, active immunity is obtained more easily, more cheaply, and also without any danger, by injecting subcutaneously 2½–3 c.c. of a well-grown bouillon culture after it has been heated to 70° for one hour. The symptoms (fever, pain) are usually moderate, and the injection is best repeated after ten days. If the protection is not absolute, and some of those injected die later of pest (1.6% instead of 24.6%), yet the majority are entirely protected or are only affected very mildly.

Special Methods for Demonstration and Culture.— 1. Not to incise non-fluctuating glandular swellings or boils of the skin for diagnostic purposes is a professional failure. In the pus from discharging ulcers, and especially in the sputum of cases of pest pneumonia, the microorganisms are found in abundance. Here a probable diagnosis is easily made microscopically from the bipolar staining.

2. To certainly demonstrate the pest bacteria in a drop of blood (more readily done in spleen- or liver-juice) after staining alone, is often impossible. It is more easily done in cultures upon gelatin at 22°, by observing the small, elevated colonies with delicate, transparent borders. Absence of spontaneous motion.

3. It is important to observe the involution forms upon 3% sodium-chlorid agar after twenty-four hours' growth.

4. The serum from cases of plague agglutinates pest bacteria.

Bacterium acidi lactici. Hüppe.

(Plate 14.)

Literature.—Hüppe, Mitteil, aus dem Gesundheitsamt II, 309. More recent literature up to 1891 is given by Scholl; Die Milch (Wiesbaden, 1891). Compare also Kayser (A. P., 1894, p. 737), where there are described 15 organisms which produce lactic acid.

Microscopic Appearance.—Short, somewhat oval rods (0.6–2 μ long, 0.4–0.6 μ broad), usually in pairs, rarely in longer chains (14, IX).

Motility and **flagella** are absent.

Staining Properties.—Stain by Gram's method, but not very well.

Requirements as to Nutrient Media and Temperature.—Grows abundantly at room and incubator temperature upon the various nutrient media. It grows better aerobically, and not at all deep in shake cultures prepared from non-saccharine media. With the addition of sugar, it grows well anaerobically.

Growth upon Gelatin and Agar.—Not essentially different from Bact. coli, very abundant, especially upon agar, and moist and slimy. Upon gelatin, delicate. In thin plate, the colonies may become 5–10 mm. in diameter (14, V).

Bouillon Culture.—Diffuse cloudiness, abundant sediment.

Potato Culture.—Somewhat widely spreading, wavy, smooth-edged growth, somewhat elevated, at first grayish- to yellowish-white, later sometimes brownish-yellow. After longer standing, bubbles arise which often are strongly refractive, and later may burst (14, X).

Milk Culture.—Compact coagulation, with expression of clear serum; a few little gas bubbles are always present.

Chemical Activities.—It forms from grape- and milk-sugar a mixture of lactic and acetic acids, and sometimes traces of alcohol, together with an abundance of gas. The lactic acid may be optically inactive fermentation lactic acid, but so far special investigations are lacking. As first observed by Hüppe, the powers of producing lactic acid and of coagulating milk are gradually lost after long cultivation upon gelatin or agar.

Upon nutrient media free of sugar, there is a slight production of indol, but none of H_2S.

Distribution.—Constantly cultivated from sour milk by Hüppe in Berlin, and by his pupils with slight modifications (consult Scholl). In Würzburg, since 1888 (compare Dissertation by Joh. Claus, Bakteriologische Untersuchung der Milch im Winter 1888–89 in Würzburg), we have never failed to find the organism in milk which had soured spontaneously and naturally, and until recently we had no doubt that it was the most important producer of lactic acid in milk, as Hüppe assumed. Milk which has soured spontaneously contains, in Würzburg, considerable quantities of volatile acid. As soon as possible the question as to the most important cause of lactic acid fermentation will be restudied in Würzburg. Compare page 224.

Demonstration and Differential Diagnosis.—As differing from Bact. Güntheri, the Bact. acidi lactici grows well upon the ordinary nutrient media, and produces gas vigorously. As regards the staining by Gram's method, variations occur. In order to bring the findings into a scheme we call the forms not stained by Gram's method Bact. lactis aërogenes (see below), and leave the question open as regards the kind of relationship existing between these "species."

Bacterium aërogenes.[1] (Kruse.) L. and N.

Synonyms.[2]—Bacterium lactis aërogenes Escherich, Bacillus aërogenes Kruse.

Literature.—Escherich, Die Darmbakterien des Säuglings, 1886, page 57.

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[1] A Bact. lactis aërogenes obtained from Král presented from 1 to 3 irregularly arranged, long flagella, and was thus, according to our ideas, a typical Bact. coli. It also produced indol very vigorously.

[2] We cannot understand how Kruse designates the Bact. acidi lactici as a variety of the Bact. aërogenes, which was described many years later. If one name is to be eliminated, according to priority, it must unquestionably be that of Bact. aërogenes.

This variety, first isolated from the milk-stools of infants by Escherich, is, according to our investigations, and according to Escherich's own statements, to be differentiated from the Bact. acidi lactici merely by the absence of staining by Gram's method[1]—a characteristic upon which no great value can be placed according to recent experiences. A further difference, which Escherich understands from Hüppe's description, that Hüppe's organism was an obligate aerobe, we cannot recognize according to our investigations as present, for as often as we isolated the Bact. acidi lactici from sour milk in Würzburg, it always produced fermentation anaerobically. We cannot place any great value upon the luxuriant, sometimes hemispheric, slimy growth upon the surface in the gelatin stab, which he likens to the growth of the Bact. pneumoniæ. Escherich has even seen exceptions.

Metabolic Products.—Alcohol, acetic acid, active lactic acid, succinic acid, and, according to Nencki (C. B. x, 82), also CO_2 and H. According to Smith, about 30%–40% CO_2, 60%–70% H. Indol is not produced.

For us Bact. lactis aërogenes is the name for a form without flagella, parallel to the typical peritrichous Bact. coli, or for a Bact. acidi lactici which is not stained by Gram's method. Transition forms certainly may exist,—compare remark 1,—but one proved to be well founded is not certainly known to us. Very closely related is the Bact. diatrypeticum casei Baumann (C. B. XIV, 494), which is widely distributed in milk, water, and soil, and causes the cavities in cheese, or perhaps aids in their formation. Composition of gas: 63% CO_2, 37% H_2. It is provided with a capsule.

We can see no final proof in the investigations of Scheffer (A. H., 1897, XXX, 291) by which he attempts to make a distinction between the two varieties dependent upon immunization and agglutination experiments, for we remember that the different varieties of the streptococcus furnish no reciprocal immunity, and that each form of the Bact. coli furnishes a serum which strongly agglutinates only the form concerned.

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[1] Würtz and Lendet find both varieties identical.

Here belong the following non-motile varieties, which ferment grape- and milk-sugar :

Bacterium cavicida Brieger. Zeit. f. phys. Ch., 8.

Bacterium neapolitanum Emmerich. Cultivated from a series of cholera cadavers in Naples and once from the blood of a cholera patient. It is not the cause of cholera. According to Buchner, the moderate vibratory motion is not purely molecular. Flagella are not known. If it possessed flagella, then it would be considered as Bact. coli. Compare Weisser (Z. H. I, 315).

Bacterium of septicemia of cats Lehm. and Neum. Cultivated from a cat which died spontaneously. Killed cats with typhoid symptoms. A more detailed description is still lacking.

Bacterium of dermatitis epidemica exfoliativa Russel (C. B. XV, 324). Unknown to us.

Bacterium caucasicum. (Kern.) L. and N.

Synonyms.—Dispora caucasica Kern. Bacillus caucasicus v. Freudenreich.

Literature.—v. Freudenreich (C. B. L. III, 47, 87, 135).

Microscopic : Rods, about 5–6 μ long, 1 μ broad, which often present small, clear, globular swellings at the ends (are not spores !). Very slightly motile.

Fresh cultures grow poorly or not at all upon gelatin or milk-sugar gelatin ; on the contrary, old cultures grow well. Upon milk-agar there develop whitish-gray, flat colonies with a somewhat jagged border due to outward projection of individual bacteria. Milk is not coagulated. Little gas-formation in milk ; grows well in milk-sugar bouillon. Growth at 22° is feeble ; 37° is the optimum.

According to Kern, it is the cause of kephyr fermentation. v. Freudenreich obtained kephyr in sterile milk most often (not always) if he mixed together four varieties : (1) The kephyr yeast ; (2 and 3) two streptococci isolated from kephyr ; (4) the Bact. caucasicum ; but also with the yeast and the two streptococci there resulted a tolerable production of kephyr.

Bacterium Güntheri. Lehm. and Neum. Günther and Thierfelder (A. H. XXV, 164).

Literature.—Günther and Thierfelder (A. H. XXV, 164). Leichmann (C. B. XVI, 826). Consult especially Leichmann (C. B. L. V, 344).

Nomenclature.—Günther and Thierfelder have not named their organism. In our first edition, published in May, 1896, we gave it the name Bacterium Güntheri L. and N. This name must stand, for also Leichmann, who had received the organism from Günther and Thierfelder, but did not especially study it, designated it by the name **Bacterium lactis acidi** for the first time, so far as we can see, in December, 1896 (C. B. L. II, 777). Aside from the question of priority, it is very impractical to introduce a Bacterium lactis acidi, together with a Bacterium acidi lactici. Besides, Leichmann has also called a longer, slender, thermophilic, non-sporulating, acid-producing variety Bacillus lactis acidi. Later than our name is also **Bacillus lacticus** Kruse. Lately Kozai has introduced **Bacillus acidi paralactici** (Z. H. XXXI, 337).

Microscopic.—Short rods, 1 μ long, 0.5–0.6 μ thick, in pairs or short chains; at the ends somewhat pointed; stains by Gram's method; non-motile, facultative aerobe. *Upon the gelatin plate:* Punctiform colonies, never more than 0.5 mm. in diameter upon medium which does not contain sugar; when sugar is added, they are a little larger, but always very delicate, and never liquefying. In the stab culture there is often scarcely anything but a deep growth. *Upon the agar plate:* Delicate transparent growth, like the finest dewdrops. *In bouillon:* Slight cloudiness when no sugar is present, marked turbidity when sugar or milk are added. *Milk:* Coagulated; reaction strongly acid. From grape- and milk-sugar pure dextrorotatory lactic acid (no other acid) is produced, but no gas. Upon potato there is a limited growth.

Distribution.—According to Leichmann, Günther, Thierfelder, and Kozai, it is found in abundance in all spontaneously coagulated milk, and is either the general producer of lactic acid or, at least, the most important for certain places and times. Yet the single fact that spontaneously soured milk contains preponderantly the long-known inactive fermentation lactic acid, shows that other varieties besides the Bact. Güntheri are concerned in the process. Compare Leichmann (C. B. L. II, 777).

Kozai (Z. H. XXXI, 337) has demonstrated for Halle that, especially at higher temperature, two varieties, which produce lactic acid, work together. They are given the names **Bacillus acidi lævolactici** and **Micrococcus acidi paralactici liquefaciens Halensis.** By this last name the necessity of the binomial nomenclature might be strikingly pointed out. Why not **Micrococcus**

halensis ? The Bac. acidi lævolactici resembles, morphologically and biologically, the Bac. acidi lactici Hüppe, but at room temperature only coagulates milk slowly (often milk becomes a thick fluid only after twelve days), while in the incubator it coagulates milk rapidly. The acid formed is levorotatory lactic acid. The coccus is provided with a thick capsule, liquefies gelatin, and forms dextrorotatory lactic acid. During the final reading of our proof-sheets, Leichmann, in a partial work, claims to find in sour milk, besides his Bact. lactis acidi, also the Bact. acidi lactici—in the layer of cream, often even in preponderating number (C. B. L. v, 344).

Special Culture Methods.—Ordinary gelatin or agar plates do not give good results because of the minuteness of the colonies. The best medium to employ is a chalk medium (see Technical Appendix) which contains grape- or milk-sugar. Upon this the colonies are surrounded by a clear halo. Also good results are obtained with milk-peptone gelatin. One pays attention to the small colonies.

Bacterium pneumoniæ. Friedländer.[1]

(Plate 15.)

Literature.—Friedländer (Fortschr. d. Med., Bd. I, 715, etc.).

Synonyms.—Pneumonia bacillus of Friedländer, capsule bacillus of pneumonia; also compare pages 227 and 228.

Microscopic Appearance.—Short rods (0.6–3.2 μ long, 0.5–0.8 μ broad), with rounded ends. When from the animal body, they present a thick gelatinous capsule, which is developed only in milk among the nutrient media.

Spontaneous motility is absent.

Staining Properties.—Stains by the usual methods even in the cold, but not by Gram's method. The capsules, which are colorless after the usual stain, may, however, be stained. (See Technical Appendix.)

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[1] The **Bact. tholœideum** Gessner is only differentiated by its effect upon mice (A. H. IX, 129). Also the **Bact. butyri colloideum** Lafar (C. B. XIII, 807), constantly present in butter, according to Lafar, appears also related, although not yet sufficiently described biologically.

Requirements as Regards Nutrient Media and Oxygen.—Grows luxuriantly upon all the nutrient media employed, both with and without oxygen.

Gelatin Plate.—(*a*) *Natural size. Superficial:* Round or roundish, moist, white colonies, with even border, usually much elevated, rarely flat, with a slimy-fatty luster. *Deep:* Roundish to whetstone-shaped, yellowish-white (15, v).

(*b*) *Magnified fifty times. Superficial:* Round colonies with smooth border, reddish to yellowish-brown, transparent only at the periphery. Sometimes there extend outward from the center rays which appear as dark brown thorns and points upon the lighter underlying part (15, vii). Usually a structure can scarcely be distinguished. *Deep:* Roundish to whetstone-shaped, smooth border, brown, opaque (15, vi).

Gelatin Stab.—*Stab:* Well developed, yellowish-white, like a string of pearls. *Surface growth:* Elevated, like the head of a nail. The gelatin is sometimes a little brownish about the puncture, but never liquefied(15, ii).

Agar Plate and Stab.—Similar to the growth in gelatin, only the colonies are perhaps still more luxuriant and moister.

Sometimes we observed in plates, instead of the roundish deep colonies, single deep veil-like spreading colonies, some of which are reproduced in Plate 15, viii.

Agar Streak.—Growth spreading moderately, whitish-yellow to gray, with a moist luster, much elevated, especially in the middle. The border is smooth, wavy, and the periphery transparent. Water of condensation is cloudy, with a slimy deposit (15, i).

Bouillon Culture.—Very cloudy, with a slimy deposit at the bottom, which upon shaking becomes homogeneous. Bouillon becomes somewhat thickened.

Milk Culture.—Not coagulated after twenty days. Abel never found milk coagulated by true Bact. pneumoniæ, but the opposite was observed by others; for example, Löwenberg (A. P., 1894, 292). Compare the observations of Denys and Martin, page 229.

Potato Culture.—Thick, moist, highly shining growth, with smooth but scalloped border, bright yellow to grayish-brown. It is gradually separated into padded, connected sections, especially at the border.

Chemical Activities.—From grape- and milk-sugar the bacterium produces abundant acid, together with CO_2 and H_2 (40% CO_2, 58% H_2, Th. Smith). P. Frankland demonstrated as fermentation products: ethyl alcohol, acetic acid, a little formic and succinic acids. It is surprising that lactic acid is not mentioned. Indol and H_2S are scanty.

Distribution.—(*a*) *Outside the body:* Cultivated by Emmerich from the foul floor of a prison.

(*b*) *In healthy organism:* Sometimes in saliva.

(*c*) *In diseased human organism:* As the cause of a few cases of pneumonia and bronchitis, then occasionally, but not very often, as the cause of inflammatory and suppurative processes in almost all the organs of the body; rarely as the cause of pyemia and septicemia. Often also found in the blood. Rarely it causes cystitis (Montt-Saavedro, C. B. xx, 171).

(*d*) *In animals:* The cause of pneumonia in horses, discovered by Schütz, is morphologically almost identical (Arch. Tierheil., XIII). Nail-head cultures usually are lacking and the growth upon gelatin is flatter. The organisms are abundant in the lungs and pleuræ, *i. e.*, especially in the necrotic parts, but sparingly in the blood. Fiedeler substantiated the findings in all points (C. B. x, 310).

Immunity and Serum Diagnosis.—Active immunization is possible; the serum causes agglutination, although the B. P. is non-motile. Landsteiner (Wien. klin. Wochenschr., 1897, 439).

Results of Experiments upon Animals.—Mice become sick after subcutaneous, more certainly after intrapulmonary injection, also after inhalation, and soon die, with the appearances of septicemia. Also guinea-pigs and dogs are susceptible, but rabbits are not.

Of the numerous closely related varieties[1] we must mention two somewhat more extensively, because they are found in typical infectious diseases of man, even though they differ morphologically from the other forms only in the insufficient characteristics already mentioned in the key to the recognition.

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[1] Also the species in the following list (capsule bacilli of authors) must be considered as forms which are identical with or closely related

Bacterium ozænæ (Abel). Lehm. and Neum.

Bacillus mucosus ozænæ (Abel, Z. H. XXI, 89); Löwenberg (A. P., 1894, 292). Paulsen: Bacterium of atrophic rhinitis (C. B. XIV, 249).

Rods of very variable length, capsule in the body often double the width of the bacillus on each side, sometimes capsules occur in milk cultures. The cultures are not different from those of Bact. pneumoniæ, only they are somewhat more fluid. The formation of gas upon potato or coagulation of milk was never observed. Sometimes marked, sometimes slight fermentation of grape-sugar. Old cultures sometimes become a little brownish, but without a brown color of the nutrient medium being produced.

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to the Bact. pneumoniæ, because, after all we know to-day, we cannot recognize, as true characteristics of species, slight differences of adaptation to a certain variety of animal, the luxuriance of growth, the imperfection of Gram's stain, or greater or less ability to produce fermentation:

Bacillus pneumoniæ Friedländer (Fortschritte der Medizin, 1883, I, 715).

Bacillus pseudopneumonicus Passet (Aetiol. der eitr. Phlegmone, Berlin, 1885).

Proteus hominis capsulatus Bordoni-Uffreduzzi (Z. H., Bd. III, 1887, p. 333).

Capsule bacillus from canal-water von Mori (Z. H., Bd. IV, 1888, p. 47).

Capsule bacillus of R. Pfeiffer (Z. H., Bd. VI, 1889, p. 145).

Capsule bacillus of Mandry (Fort. d. Med., Bd. VIII, 1890, 205; C. B. VII, 570).

Capsule bacillus of Kockel (Fort. d. Med., Bd. IX, 1891, 331).

Bacillus capsulatus mucosus Fasching (C. B. XII, 304).

Capsule bacillus of v. Dungeren (C. B. XIV, 541).

Capsule bacillus of Marchand (C. B. XV, p. 428).

Capsule bacillus of Nicolaier (C. B. XVI, p. 601).

Capsule bacillus in keratomalacia of Loeb (C. B. X, 369; much literature).

Bacillus sputigenus Pansini (C. B. IX, 566). Somewhat more pronounced differentiation.

Bacillus sputigenus crassus Kreibohm (C. B. VII, 312). (Stained by Gram's !)

Bacillus aërogenes sputigenus capsulatus Herla (C. B. XXV, 359).

Bacillus capsulatus chinensis A. Hamilton (C. B. L. IV, 230). (Always forms capsules; literature résumé.)

The organism occurs regularly in ozena (foul), but also in pure atrophic rhinitis without odor. The significance of the organism in the production of the ozena is therefore very questionable, just as is the significance of the pseudodiphtheria bacillus, which is often simultaneously found. Jurasz and Hecht go so far as to question the significance of bacteria in ozena, and speak of a trophic neurosis of the nose with a putrid secretion. Compare Hecht (Münch. med. Wochenschr., 1898, No. 7, 198).

Mice die in from one to four days after subcutaneous inoculation; rats and guinea-pigs are more difficult to infect, and rabbits are immune.

Bacterium rhinoscleromatis v. Frisch.

Literature.—Paltauf (C. B. I, 236); Bender (C. B. I, 563); Dittrich (C. B. II, 89, 433); Babès (C. B. II, 617); Dittrich (C. B. V, 145); Zagari (C. B. VI, 450). It behaves in all essential properties like the Bact. pneumoniæ, yet many authors (Dittrich, Zagari) find it stains by Gram's method, but others do not. The growth in the gelatin stab shows the nail-head form, is more of a transparent gray, and not quite so white as in the Bact. pneumoniæ. Further differences can not be found even by the vigorous advocates of a difference between the Bact. rhinoscleromatis and Bact. pneumoniæ. According to Paltauf, milk is coagulated; according to Abel, it is not. It is found in all cases of typical rhinoscleroma (infrequent, hard, round-celled tumors of the nose, partly subcutaneous, partly submucous; more rarely in throat and larynx) and claimed to be the cause of the same. In animal and human experiments a reproduction of rhinoscleroma has never succeeded. De Simoni doubted that the organism is different from the above members of the group of Bact. pneumoniæ, and, above all, that it is the cause of rhinoscleroma (C. B. XXV, 625). The constancy of the occurrence of the organism in all cases of rhinoscleroma examined bacteriologically remains as an incontestable, significant fact. Dittrich found the organism generally to be scarcely at all pathogenic; others observed mice to be about as susceptible to it as to the Bact. pneumoniæ, and guinea-pigs less so.

Critical Remarks Regarding Bact. acidi lactici, aërogenes, pneumoniæ, rhinoscleromatis, and ozænæ.

These varieties are, as appears from the description, at least closely related, and only to be differentiated by biologic characteristics which are known to be variable. Besides, Denys and Martin (La Cellule, IX, 1893, p. 261; C. B. XVI, 127), by repeated cultivation of pure cultures in milk, have brought the Bact. pneumoniæ, from three different sources, to a condition where it coagulates milk with the greatest energy, and also produces gas from milk-sugar. Inversely, after being grown for eleven months upon gelatin the power of breaking up grape- and milk-sugar with liberation of gas was lost, the cultures then growing thin and delicate upon potato, but still coagulating milk. They thus resembled the Bact. Güntheri, but it is stained by Gram's method.

For us, consequently, all the above-mentioned forms are botanically only biologically characteristic adaptation forms of the same organism, which must come under the oldest name of Bacterium pneumoniæ Friedländer. For practical purposes we will, as formerly, differentiate the "varieties," but we must be conscious of their close relationship and of the possibility, in part proved, of their being converted into one another. These conclusions agree essentially with the statements of Kruse and Wilde (Flügge-Kruse's Lehrbuch, III. Aufl., p. 336, and Wilde, Diss., Bonn, 1896), founded upon special studies. They have made observations regarding the variability of flagella, especially in this group, which correspond exactly with what occurs in other groups, as we know from our own observations or from what is found in literature, so that the relationship with the colon group stands out yet more strongly. We have failed to distinguish, like Kruse, a Bact. coli immobile, yet the less moist forms of Bact. aërogenes, according to our own and Kruse's judgment, can not be distinguished from the Bact. coli except by a lack of motility.

Bacterium lactis viscosum. (Adametz, C. B. ix, 698.) Lehm. and Neum.

Resembles the Bact. pneumoniæ both macroscopically and microscopically. Upon the gelatin plate it often appears as elevated droplets. Non-motile, with capsules, staining by Gram's method. The surface growth in the gelatin stab is wide-spread but not very luxuriant; upon agar and potato abundant, white, tenacious. Neither grape- nor milk-sugar is fermented; little indol and no H_2S are formed. Milk and bouillon gradually become viscous, slimy, and may be drawn out in long threads. The milk is not coagulated, and is feebly alkaline; bouillon becomes very cloudy. The slime is a carbohydrate, which originates from the capsules of the bacteria. In our culture, obtained from Král, nothing was to be seen of the spore-formation which Zimmermann claims to have seen. The organism was discovered by Adametz as an important enemy of the butter industry, the cream becoming slimy and the butter obtained therefrom spoiling and becoming soft and pale. Found by Zimmermann in water. Leichmann's bacillus, which is somewhat thermophilic, does not form spores, and ferments sugars, appears different (C. B. XVI, 122).

The **Bacterium Hessii** Guillebeau (C. B. XI, 439) is different. It is actively motile, liquefies gelatin, and forms no capsule. It likewise makes milk tenacious and no spores are described. In the same place may be found some further statements regarding varieties which render milk tenacious. Compare also Micr. Freudenreichii Guil. (p. 174).

Bacterium Pflügeri[1] (Lassar) Ludwig. Bacterium phosphorescens. Bernh. Fischer (Z. H. ii, 92).

Literature.—Ludwig (C. B. II, 372); K. B. Lehmann (C. B. V, 785); Beijerinck (C. B. VIII, 616, 651); Katz (C. B. IX, 157).

Microscopically, short, plump rods, single or in pairs. Also spherical and short oval forms occur. Striking involution forms appear in old cultures. There are neither spontaneous motion nor flagella. Beijerinck claims to have observed spontaneous motion in sea-water. Facultative anaerobe, but does not emit light when air is excluded. The addition of 3% of sea-salt is favorable. Optimum at 20°, maximum at about 39°, minimum at 0°. Upon gelatin and agar it is indistinguishable from the Bact. acidi lactici ; once we obtained upon gelatin plates colonies exactly like those in Plate 19, I, with most peculiar outgrowths. Older gelatin and agar cultures exhibit a tendency to become yellowish and yellowish-brown. Gelatin is never liquefied. Potato cultures are yellowish, moist, sometimes with gas bubbles. Grape- and milk-sugar and maltose are converted into acid, accompanied by abundant formation of gas. Milk is coagulated.

The emission of whitish, greenish light is intense if oxygen is admitted as long as the cultures are frequently transferred to fresh nutrient media containing salt ; but if this is omitted, the emission of light is soon lost. For a time the photogenic function may be regenerated by transplantation upon salt (herrings) gelatin, but it is permanently lost in time if the bacteria are cultivated upon ordinary media with infrequent transfer. Concerning the photogenesis, compare page 57. A few drops of phosphorescent bouillon culture may give a milky luster to a liter of sea-water.

Neither the bacterium nor its metabolic products in small amounts are harmful. It lives in the northern seas, causes occasionally phosphorescent sea, more often phosphorescence of fish, meat, etc.

The **Bacterium of Giard** (C. B. VI, 645 ; VIII, 177), which is pathogenic for crawfish, and makes the living, inoculated animal phosphorescent, appears, from the incomplete description, to be similar. Phosphorescent gnats (mycetophila), observed as rarities in Germany, must owe this property to bacteria. Henneberg (C. B. XXV, 649). The phosphorescent bacillus described as **Photobacterium javanicum** Eykmann (C. B. IX, 656) is plump and motile. Regarding a second group of photogenic micro-organisms, see under Vibrio albensis Lehm. and Neum.

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[1] Beijerinck distinguished B. phosphorescens from B. Pflügeri by biologic characteristics.

Bacterium typhi. Eberth, Gaffky.

(Plates 16 and 17.)

Ordinary Names.—Typhoid bacillus, Bacillus typhosus Kruse-Flügge.

Literature.—Exhaustive list of literature (689 in number) by Lösener (A. G. A XI, 207).

Microscopic Appearance.—In organs usually short, rather plump rods (1.0–3.2 μ long and 0.6–0.8 μ broad); much less often found in short chains. In cultures all forms, from short rods to long threads occur, threads being especially well developed upon potatoes of acid reaction. The shining polar bodies are not spores (see below). According to Leo Müller, however, the abundance and regularity of the occurrence of these bodies upon feebly acid nutrient media distinguishes the Bact. typhi from the B. coli (A. K., I. Band, Heft I, 1894) (17, VIII).

Spontaneous Motion and Flagella.—Active motion of the short rods; in threads a very beautiful snake-like motion is seen. The flagella are long and tortuous and are located all about the surface of the bacteria in numbers of 8 to 14 (17, IX, X).

Staining Properties.—Not by Gram's method.

Requirements as Regards Nutrient Media, Temperature, and Oxygen.—Grows best as aerobe, also as anaerobe, and fairly well in CO_2. Grows well upon all nutrient media employed, and bears acid well. Optimum about 37°. Upon non-albuminous nutrient media, such as Uschinsky's and similar combinations, it grows but scantily. According to Proskauer and Capaldi, it, in contrast to the Bact. coli, does not require the amido and ammonium nitrogen of the body for its growth.

Gelatin Plate.—

(*a*) *Natural size. Superficial:* At first small, yellowish, punctiform colonies, becoming, in a short time, roundish, irregularly notched or delicately lobulated, and shining. The periphery is clear, transparent gray, the center whitish, opaque, grayish-yellow, sometimes slightly elevated. *Deep:* Punctiform, later roundish or usually whetstone-shaped, yellowish (17, III).

(*b*) *Magnified fifty times. Superficial:* Up to forty-eight hours the colony is entirely colorless and transparent. The border is smooth and lobed. The surface presents wavy elevations, and numerous branching, strongly reflecting, whitish, winding lines, which look like incisions or scratches. The colony then appears fairly homogeneous, grayish-yellow, with white, curved lines and bands extending from the center, between which are to be seen concentrically arranged lines. These lines are the expression of folds in the colony (17, I). When more highly magnified (× 150) (17, II), indistinct parallel curved lines may be seen. Not infrequently the superficial colony is thicker, and then there occurs, instead of the windings, etc., in the yellowish almost opaque appearing colony, only an indistinct development of figures resembling hen-tracks. (Compare 19, VI.) All the forms reproduced in Plates 18 and 19 of the Bact. coli may also occur; also spirals and tail-like appendages, as in Plate 19, I. *Deep:* Round to roundish colonies, bright yellow, homogeneous, delicate, shaded with gray, with even borders (16, VII; compare also 19, V).

Gelatin Stab.—*Stab:* Thread-like, slightly granular, whitish-gray (16, III). *Surface growth:* Thin, white, grayish-green, iridescent, extremely transparent, roundish, notched, with a dry luster, not elevated, extending to the glass (16, IV).

Gelatin Streak.—Somewhat spreading, white and thin growth, as on the surface of the puncture.

Agar Plate.—

(*a*) *Natural size. Superficial:* Irregular, roundish, grayish-white, shining, slightly elevated colonies. *Deep:* Punctiform, gray (17, IV).

(*b*) *Magnified sixty times. Superficial colonies:* Round or roundish, smooth border, bright yellowish, becoming darker toward the middle, finely to coarsely punctated and transparent at the edge; from the center usually dark yellow, winding or jagged lines extend outward; morulæ are rare (17, VI). *Deep:* Roundish to whetstone-shaped, border smooth or rough, brownish-yellow, opaque, without internal markings or finely granular (17, V).

Agar Stab.—*Stab:* Thread-like, sometimes a little granular, gray (16, I). *Surface growth:* Irregularly roundish, with an almost even border, whitish-gray, with an oily luster, soon extending to the wall of the tube. Later it becomes yellowish-gray (16, II).

Agar Streak.—Moderately spreading growth, wavy, with a smooth edge, whitish-gray, shining, sometimes appearing to be transparent in many places because of porosity. The water of condensation is clear with slight precipitate (16, V).

Bouillon Culture.—Cloudy with abundant sediment which is homogeneously distributed upon shaking.

Milk Culture.—Milk is not coagulated even after standing for weeks. In spite of active multiplication, only very little acid is formed.

Potato Culture.—From the line of inoculation the growth spreads quite widely as an extremely delicate, moist, often almost entirely invisible layer (17, VII), which, when touched with a platinum needle, may sometimes be drawn out into delicate slimy threads. This was first described by Gaffky (Mitt. a. d. G. A. II, 372) as characteristic, and was long held to be an important specific characteristic, but it is not present in many cultures. Other cultures, which grow typically upon acid potatoes, present, at least upon alkaline varieties or alkalinized portions, an atypical, luxuriant, grayish, white, or brownish-yellow growth, which sometimes is moister, sometimes drier, often not spreading much, and resembling the B. coli. (Compare 18, IX.)

No Spore-formation.—The formations which were formerly held to be spores, and which appear especially upon faintly acid potatoes, are of two varieties, as first pointed out by H. Buchner (C. B. IV, 353). In unstained bacteria refractive polar bodies may be mistaken for spores, but they stain especially easily with anilin dyes (quicker than the bacteria) and do not impart any increased resistance to the bacteria. In heated and stained preparations there occur vacuoles which have a resemblance to spores because of their form and size, and from not being stained by the ordinary methods, but such a vacuole is never stained by the special stain for spores. According to H. Buchner, these roundish vacuoles are located especially in the ends of the rods; according to Leo Müller, especially in the middle, while the stained masses occupy the poles. The Bact. coli shows much more irregular and very inconstant vacuole formation.

Resistance.—

(*a*) *Against drying:* They tolerate preservation in the dry condition for months; according to Uffelmann, in soil and clothing even, for one or two months. They do not withstand such a complete drying as is necessary in order to reduce to dust. Germano (Z. H. XXIV, 403). Compare also Ficker (Z. H. XXIX, 1).

(*b*) *Cold and heat:* Janowski (C. B. VIII, 167, 417, 449). They withstand cold well.

(*c*) *In manure and feces:* Over a week (Gärtner).

(*d*) *In water:* From a few hours to many days. Compare page 40.

(*e*) *Chemical disinfecting agents:* Compare Köhler (C. B. XIV, 89).

The duration of life in the human body may be very considerable. They have been demonstrated by Sahli in pleural exudate fifty days after the beginning of the disease, and by Hintze in the pus of periostitis ten months after a case of typhoid fever.

Chemical Activities.—There is no production of pigment nor odoriferous substances. It reduces solutions of litmus, converts nitrate into nitrite, and gradually leads to a disappearance of nitrite. They form levorotatory lactic acid from grape-sugar (feebly from milk-sugar) and no visible gas bubbles from any carbohydrate (verified by Buchner, A. H. III, 425, 1885). They form H_2S strongly. Indol is not produced. The cultures are rich in toxins; the germ-free filtrates are actively pathogenic.

Distribution.—

(*a*) *Outside the body:* So far, in a few cases in water and soil which have come in contact with typhoid dejecta. Recently demonstrated by Lösener in five instances in specimens of soil, portions of cadavers, and stools, where there was no suspicion of the presence of typhoid bacteria. Similar results were obtained by Remlinger and Schneider (H. R., 1896, 743).

(*b*) *In healthy body:* So far, never.

(*c*) *In diseased human body:* In cases of typhoid fever, as the cause of the disease. Cultures are obtained with greatest certainty from the spleen and lymph-glands, in which the bacilli are always distributed in small clumps. Often, also, it may be successfully demonstrated in the blood (blood from the heart, veins, rose-spots); but, to be sure, it is so often not found that the importance of the blood examination for substantiating the clinical diagnosis is not great. Kühnau found the typhoid bacillus in the blood from the veins of the arm in 10 out of 41 cases (Z. H. xxv, 492). Neufeld obtained very good results in the blood from rose-spots by making use especially of fluid nutrient media (Z. H. xxx, 498). It has also been very frequently demonstrated in the kidney, liver, and bile (Chiari), and especially in the urine (H. Neumann, C. B. VIII, 80). Regarding the occasional presence of enormous numbers of typhoid bacilli in urine, see Petruschky (C. B. XXIII, 577). Statements regarding successful cultivation from typhoid stools are comparatively rare (compare p. 237). The typhoid bacterium can itself cause the various complications in the clinical picture of typhoid. It has been demonstrated with certainty as the only cause of cases of serous and suppurative inflammations of the spinal cord, of the brain and its membranes, of the lungs and kidneys, and in the erysipelatous, phlegmonous, and suppurative processes of typhoids (in bones, skin, testicle, lymph-glands, parotid, thyroid, spleen, etc.). The pyogenic function of the B. typhi is no more contested, and has also been demonstrated by experiments upon rabbits. However, in many (the majority?) cases mixed infection with Micrococcus pyogenes, Streptococcus pyogenes or lanceolatus, etc., must be held responsible for the complications.

Results of Experiments Regarding Pathogenic Action in Animals.—According to the fairly universal view in Germany at present, the production of an infectious disease, analogous to typhoid fever in man, has never been successfully accomplished in any animal by any mode of infection. As a rule, bacteria introduced subcutaneously rapidly die, at least, do not multiply, and the injuries resulting may be produced in a similar manner by filtered cultures. Thus, the results are due to intoxication and not to infection. (Sirotinin, Z. H. I, 465.) Also Petruschky favors the idea of an intoxication rather than an infection (Z. H. XII, 261).

Chantemesse and Vidal (A. P., 1892, 755) and Sanarelli (A. P., 1892, 721) were able, on the contrary, to so increase the virulence by all sorts of artificial means that they obtained varieties which are truly pathogenic for animals. Chantemesse (H. R., 1897, 1103) was even able to produce sickness in rabbits and monkeys by highly virulent cultures introduced into the stomach, and the animals died with typhoid symptoms (clinical and anatomical). Thus the Bact. typhi becomes acclimated to the animal body.

Special Methods for the Demonstration of the Bact. typhi.

It is usually easy to cultivate them from the spleen and lymph-glands of a fresh typhoid cadaver; still, not infrequently more colonies of the Bact. coli are obtained than typhoid. The case is different when the bacteria are to be sought for in water, feces, etc. The fact that the demonstration of the Bacterium typhi when in mixtures with other bacteria appears to be very difficult for all investigators[1] has led to numerous suggestions to replace the simple gelatin plate method by better ones. A great distrust is aroused against all of these suggestions, since every new author criticizes the suggestions of his predecessor and usually discards them.

The two principal methods which have been employed are:

1. *Preliminary Culture.*—The suspicious water is placed in nutrient media which contains an antiseptic, and kept twenty-four to forty-eight hours in the incubator. Water bacteria, especially a number of liquefying varieties, die, while the Bact. typhi and coli, which are more resistant to disinfectant agents, multiply in the incubator. Unfortunately, the rapidly growing forms of Bact. coli, besides Bact. vulgare, streptococci, and oïdium, multiply more intensely than the Bact. typhi, and when plates are prepared

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[1] An idea of the difficulty is given by the fact that many authors were not able at all to isolate typhoid bacteria from typhoid stools, and that Nicolle, Grimbert, and Chantemesse declare it to be impossible to recover typhoid bacteria from water, containing abundant Bact. coli, to which they had been added.

from the preliminary culture, almost with absolute certainty many coli forms are obtained, but also, according to most of the critical writers, much fewer typhoid bacteria than were in the original fluid (Lösener).

2. *The direct preparation of plates from gelatin which contains materials interfering with growth:* phenol, hydrochloric acid, methyl violet, potato juice, etc. Lösener, who has tested all these methods, recommends the following as the only useful one: Plates are prepared directly upon gelatin containing 0.03 to 0.05% phenol. The plates are best prepared, according to Kruse, by inoculation upon the surface (Tech. Appendix). Upon this carbol-gelatin the colonies of Bact. typhi and coli grow in the usual manner; many others, especially liquefying varieties, are, on the contrary, greatly retarded. From all colonies resembling typhoid inoculations are made into liquefied 2% grape-sugar agar (about a dozen tubes) and the shake cultures thus prepared are placed in the incubator. The tubes in which there is no fermentation are studied further, as indicated on page 239.

Almost simultaneously with Lösener, Elsner studied, in Koch's Institute, the methods for the ready demonstration of typhoid bacteria by means of special nutrient media, and instead of the potato-gelatin of Holz,[1] which had given unsatisfactory results in the hands of many writers, he recommended a new feebly acid potato-gelatin containing 1% iodid of potassium. (See Tech. Appendix.) (Z. H. XXI, 25.)

According to Elsner, scarcely any bacteria except Bact. typhi and coli grow upon his nutrient medium, the liquefying varieties not at all. Bact. coli grows very well, and after twenty-four hours presents already perfectly developed colonies.

In contrast to this, the Bact. typhi grows very slowly; after twenty-four hours the colonies are scarcely visible with low magnification, and after forty-eight hours they appear as small, clearly shining colonies, like water drop-

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Special Differential Diagnosis of the Bact. typhi, Especially from the Bact. coli.

The following peculiarities must all be demonstrated:

1. Rods, short to thread forms; active motility; abundant, long, peritrichous flagella; not stained by Gram's method.
2. White film upon gelatin which is not liquefied.
3. No formation of gas from grape- or milk-sugar in a shake culture.
4. Uniform cloudiness of sugar bouillon in fermentation tubes without formation of gas. No formation of acid from milk-sugar, abundant from grape-sugar.
5. No coagulation of milk.
6. Indol not produced in peptone solution.
7. Finally, Lösener places value upon the demonstration by means of cultures in Petruschky's litmus whey (at 37°) that the questionable typhoid bacterium in about forty-eight hours does not produce more than 3.0 c.c. of decinormal acid from 10 c.c. of milk, while the coli bacteria form more than 8 c.c.[1]
8. Marked agglutination by specific serum (see below).
9. Of less value in the diagnosis are: (1) The microscopic appearance of the gelatin plate, as it may be almost identical with the Bact.

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[1] Upon all these points a very satisfactory uniformity has been reached. To be sure, the uniformity depends in part upon an agreement, which is, that all those bacteria which do not present these peculiarities of the typical typhoid culture are simply declared to be different from typhoid, under the assumption that the typhoid bacterium does not vary. How little probability this assumption possesses in the face of the enormous variability of the closely related Bact. coli, requires no discussion.

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coli. (2) The delicate growth upon potato, since there are typhoid bacteria which grow as luxuriantly as Bact. coli. In order that a potato culture may be of diagnostic value, two pieces from the same potato must be placed in a dish and inoculated, one with the culture in question, the other with a certain typhoid culture (Germano and Maurea). According to these authors, with whom Lösener agrees, a deviation from the growth of true typhoid bacteria upon the same potato is sufficient to exclude a diagnosis of typhoid. (3) Growth upon nutrient media to which are added antiseptic substances (phenol, formaldehyd, acids, etc.). The Bact. coli always tolerates these somewhat better than the typhoid bacterium.

The Diagnosis of Bacterium typhi is excluded:

If one of the following properties is demonstrated:

1. Absence of motility, flagella absent or located at the pole, typical spores, staining by Gram's method.
2. Absence of growth at body temperature.
3. Coagulation of milk. Formation of gas in grape-sugar agar or fermentation tubes.
4. Liquefaction of gelatin.

A beautiful example of a thorough differential diagnosis between mud and typhoid bacteria is given by Houston (C. B. XXIV, 518).

Serum Diagnosis of Typhoid.[1]

In doubtful cases the typhoid diagnosis may very often be verified by the serum test. Since we have been acquainted with the Gruber-Durham agglutination reaction in vitro, almost always this is employed instead of R. Pfeiffer's more detailed reaction in the abdominal cavity of the guinea-pig. Cultures upon slanted agar, eighteen to twenty-four hours old at 37°, are used for the test, and of such a culture 2 mg. are finely divided in 0.5 c.c. of bouillon (p. 105).

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[1] If one has no immune serum, still, according to Laschtschenko, he may differentiate the Bact. typhi from the Bact. coli in the following manner (H. R., 1899, No. 3): Several test-tubes, each containing 2 c.c. of fresh defibrinated rabbit's blood, obtained by venesection, are provided. To these are added two drops of a dilute suspension of the culture in question. The suspension is prepared by mixing 1 loopful of an agar culture with 10 c.c. of bouillon, and then diluting 0.5 c.c. of this with 9.5 c.c. of bouillon. In the case of Bact. coli which have not been cultivated too long, the bacteria are never dead in six to seven hours, and usually are much more numerous, while the Bact. typhi (ten cultures!) were always much less in number, no matter whether the culture had been isolated for a short or long time.

1. *Testing Doubtful Cultures by Means of Known Typhoid Serum.*—Active serum is prepared, according to R. Pfeiffer, as follows (compare also Fodor and Rigler, C. B. XXIII, 930):

A rabbit weighing from 1½ to 2 kilos is injected subcutaneously with a suspension of the bacteria from three slanted agar cultures of Bact. typhi, twenty-four hours old, the suspension having been kept in a water-bath for one hour at 65° before injection. Ten days after the injection the animal is bled into a tall narrow glass cylinder, and after the blood has stood twenty-four hours in an ice-box, clear serum is obtained.[1] (See p. 105 concerning this and the dilution of the serum.) Such a serum agglutinates true typhoid bacteria in dilutions of from 1 to 30, to 1 to 100 or more. By means of a series of tests the limits of the value of the reaction are determined. The organisms to be diagnosed must also be agglutinated by a similar dilution. If it should be affected much more feebly (*i. e.*, only with a higher concentration: for example, 1 to 20) than the standard culture, this may depend upon the greater virulence of the first, because the greater the virulence, the more easily the effect is produced. If, in spite of lower virulence, the effect is much less than in the standard culture, then the bacteria in question are not typhoid bacteria.

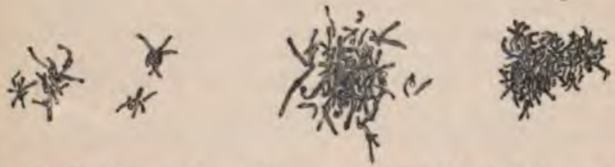

Fig. 18.—Smaller and larger clumps of agglutinated Bacterium typhi.

2. *Testing the Serum of Patients Who Are Suspected of Hav-*

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[1] Immune serum with the addition of 0.5% thymol is very stable, but also serum may be absorbed by filter-paper in certain quantity and each time a test is to be made a piece of the paper may be added to diluted typhoid bouillon (Richardson, C. B. XXI, 445)

ing Typhoid with Known Typhoid Bacteria.—The test consists in the demonstration that the serum, properly collected and diluted,[1] according to the instructions on pages 104 to 110, agglutinates typhoid bacteria from a twenty-four hours' agar culture inside of an hour at 37°, when the dilution is 50 times; or even, if possible, when diluted 100, 500, or 1000 times.

Following exactly the instructions on page 105, different tests are made with varying strengths of serum and the limit of the activity of the serum determined. If the serum does not cause agglutination in dilutions higher than 1 to 50, then no decision is possible, for serums which are active when thus diluted occur without typhoid fever being present or having previously occurred (25% of healthy persons furnish serum which is active in dilution of 1 to 10). On the other hand, the absence of agglutination does not absolutely prove that typhoid fever does not exist, especially at the commencement of the disease, for before the third week the reaction is not so very rarely absent; after the third week, it is lacking in only 1% of the cases (compare Bieberstein, Z. H. XXVII, 347). The diagnosis of typhoid is most certain when, during the course of the disease, the reaction having been previously absent, it develops with increasing vigor (v. Leube). For many details and the entire literature, consult Kasel (Verhandlungen der phys.-med. Gesellschaft in Würzburg, XXXII, No. 6, 1899); in abstract, Kasel and Mann (Münch. med. Wochenschr., 1899, No. 18, 581).

Not infrequently coli bacteria are influenced more strongly than typhoid bacteria by serum from typhoid patients, and this is explained upon the entrance of coli bacteria through the intestinal ulcers during the typhoid, etc., thus producing a mixed infection (Stern, Berl. klin. Wochenschr., 1897, 225). Thus the serum of patients (in distinction to animal serum) can be employed as a test for typhoid bacteria only when it has been previously found entirely or almost without effect upon known Bact. coli.

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[1] The proposal to employ blood instead of serum in the diagnosis of typhoid is often made and has been found practical. With a pipet 0.1 c.c. of blood is placed in a small graduated cylinder and diluted to 2 c.c. This blood mixture is tested and corresponds in its effects to a serum diluted 40 times. Compare Babucke (C. B. XXIII, 1092). If one possesses a Thoma-Zeiss counting apparatus for leukocytes, 0.5 c.c. of blood may be drawn into the melangeur and then 10 c.c. of water. Of the mixture, a drop is mixed with a drop of suspension of bacteria. Thus the dilution becomes 1 to 40. (Rostoski, Münch. med. Wochenschr., 1899, 209.)

Regarding the relation of the Bact. coli to the Bact. typhi there has been much work done and still more written. Objectively considered, the case is that numerous things speak for the probability that the Bact. typhi may originate from the Bact. coli by a loss of certain zymogenic and the acquisition of pathogenic properties, but the possibility of such a change is not proved by any experimental investigation. We may believe what we will, but for the present the Bact. coli and Bact. typhi are two different organisms. Nothing pertinent to the question is afforded by the demonstration, coming from various sides, that the Bact. coli may lose its ability to produce indol and coagulate milk. Also, the circumstance that Peckham (Jour. f. ex. Med., 1897, II, 549; C. B. XXIII, 986) was able to stimulate typhoid bacteria to vigorous production of indol, and that cultures absolutely like typhoid are found which present no agglutination with typhoid serum, do not certainly prove the transformation of one kind into the other, but only point to an extraordinarily close relationship.

Bacterium coli (Escherich). L. and N.

(Plates 18 and 19.)

Synonym.—Bact. coli commune Esch. (Darmbacterien des Säuglings, Stuttgart, 1886). Compare page 250 and forward.

Literature.—Kiessling, Sammelreferat (Hygien. Rundschau, 1893, III); Lösener (A. G. A. XI, 207); Germano and Maurea (Ziegler's Beiträge, XII, 494; C. B. XV, 60); Tavel and Lanz (C. B. XIV, 705); Von Stöcklin (C. B. XVI, 130); Gordon (Jour. of Path. and Bact., IV, 438).

Common Names.—Colon bacillus, Coli bacillus, "Coli Escherich."

Microscopic Appearance.—According to the nutrient medium and the age of the culture, the B. coli occurs as almost isodiametric, oval forms, or (and as a rule) as short rods, 2 to 4 μ long and 0.4 to 0.6 μ broad; more rarely in the form of shorter or longer threads. The ends are rounded; not infrequently two rods lie together in pairs; also chains of bacilli occur. In unfavorable conditions (old potato cultures, soda bouillon) there occur readily staining polar bodies at the ends of the rods, while the middle remains unstained. Young rods are always actively motile. Concerning non-motile forms, see under Bact. aërogenes (19, VIII, IX).

Staining Properties.—Easily stained, even with cold solutions, by ordinary methods. It is not stained by Gram's method.[1]

Form and Arrangement of the Flagella.[2]—Most authors, like ourselves, have found the flagella similar to those of the Bact. typhi, but a little less numerous—*i. e.*, 4 to 8 peritrichous, long, slightly wavy flagella. Stöcklin has found very great variation in individual "varieties" of Bact. coli in this respect; some correspond to the description given above, a greater number possess 1, 3, or 5 flagella, a few generally only a single flagellum at the end. In the very painstaking work of Remy and Sugg the flagella of the B. coli are described as somewhat shorter than in the Bact. typhi, and very fine (C. B. XIV, 70). We cannot entirely confirm this, for among the different varieties (about twelve) which were stained by us there were some with very long flagella. Sometimes the flagella extend outward from a colorless capsule.

Relation to Oxygen.—Grows best as aerobe, especially upon nutrient media containing sugar; anaerobically it does not grow quite so well, and still more poorly when sugar is absent. It grows also in CO_2, but not quite so well.

Requirements as Regards Temperature and Nutrient Media.—It grows rapidly at room temperature and very well at 37°, is satisfied with the various nutrient media, tolerates quite strong acid reaction, but in nutrient media containing sugar it not infrequently produces more acid than it can tolerate, and so dies out. It grows well in nutrient media which contain no albumin.

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[1] The statement of Alexander Schmidt (C. B. XIII, 761) that by cultivating upon nutrient media containing fat the Bact. coli acquires the property of staining by Gram's method, we could not verify, and no more could Jacobsthal (H. R., 1897, 849).

[2] We group provisionally the forms which have only one or a few polar flagella, instead of a large number of peritrichous flagella, as special "forma polaris" Lehm. and Neum. (compare p. 252). The non-motile forms we place under Bact. aërogenes.

Remarks Regarding the Macroscopic Growth of the Bact. coli.—All closely observing recent authors (Dunbar, Ferrati, Lösener, etc.) state that the Bact. typhi and Bact. coli cannot be differentiated with certainty by means of their cultures, but only that generally the coli bacteria grow more luxuriantly upon the various nutrient media. The color is white, often, in thick growths, becoming gray or yellowish-white.

Gelatin Plate.—(*a*) *Natural size:* Like the Bact. typhi, except that here the moist, opaque forms (not uncommonly somewhat raised above the medium in the form of drops) are more frequent than the thin, delicate, iridescent growths, which are the rule in the Bact. typhi (19, II).

(*b*) *Magnified seventy times:* Not to be differentiated with certainty from Bact. typhi (compare 17, I), but on account of the greater thickness of the film, the beautiful striking system of furrows is rarely well developed in Bact. coli (19, III, IV, and VI). We have often observed the deep colonies, which, as a rule, are roundish or whetstone-shaped (compare 17, V, and 15, VII; both types are very frequent), present wonderful, drawn-out, lobulated, tailed forms, which remind one of the zoogleæ of Bact. vulgare, and for which we can make only high temperature (softness of the gelatin) responsible (19, I). Similar formations have since been described by W. Rosenthal (Deut. Arch. klin. Med., LV, 513). Compare also the complete representation of Klie, who observed and represented such forms of colonies of typhi and coli bacteria, especially upon soft nutrient media, containing little gelatin (C. B. XX, 49).

Gelatin Stab and Streak.—Like Bact. typhi, only somewhat thicker, more opaque, and more rapidly growing. Never liquefying (18, I, II).

Agar Plate.—Colonies exactly like Bact. typhi, only usually somewhat thicker and moister. Magnified seventy times, the deep colonies often appear somewhat rough and knobby (18, VI), the superficial usually roundish, finely punctated, almost structureless, and opaque; at other times they are finely lobulated, with markings like a mulberry.

Agar Streak and Stab.—Like Bact. typhi, often more luxuriant (18, III, IV, V).

Bouillon Culture.—Cloudy, with a moderate, slimy precipitate, which upon shaking rises up and becomes homogeneously distributed. Sometimes there is a distinct pellicle formed on the surface of the bouillon.

Milk Culture.—Milk is usually rapidly coagulated; more rarely, slowly. In connection with the ability to break up milk-sugar, coagulation of milk is never absent. Regarding non-coagulating forms, see under Bact. choleræ suum.

Potato Culture.—Growth with a wavy outline, at first yellowish-white to grayish-yellow, later pea-yellow to yellowish-brown and grayish-brown, partly flat, partly much elevated, usually with a moist luster, less often dry and dull. The potato in the region of the growth is usually discolored (18, IX). Rarely the Bact. coli produces a delicate, almost invisible potato growth resembling that of Bact. typhi.

Resistance to various injuries is about like that of the Bact. typhi. It is even more resistant to acids, formalin, and other chemicals. According to Walliczeck, it bears drying poorly (C. B. XV, 947).

Chemical Activities.—

(*a*) *Chromogenesis:* Only upon potato and always moderate (yellowish-brown).

(*b*) *Odoriferous and gustable substances:* Uncharacteristic, ill-smelling substances are developed upon agar and gelatin, but especially upon potato cultures.

(*c*) *Gas and acid production from carbohydrates:* Grape- and milk-sugar are fermented, with the production of a mixture of acetic, formic, and lactic acids. According to Oppenheimer, there are formed 70% volatile and 30% non-volatile acids, and some iodoform-forming substance (alcohol). Many cultures ferment cane-sugar also. With this fermentation there occur abundant CO_2 and H_2 in varying proportion; we found about one-fourth CO_2, the rest being H and some N, but no marsh-gas. According to Péré (A. P., 1893, 737), three different Bact. coli formed levorotatory lactic acid from nutrient media with grape-sugar, which contained peptone as a source for nitrogen, just as is done by Bact. typhi. But if ammonia was the source of the nitrogen, then only the Bact. typhi and one Bact. coli isolated from man produced levorotatory lactic acid in a remarkable manner; both the other coli cultures (from cheese and animal feces) produced dextrorotatory lactic acid.

(*d*) Vigorous production of H_2S from peptone; usually abundant indol. We have never failed to find traces of indol.

Karplus found, in the urine of a patient, an organism resembling the typhoid bacterium, which produced H_2S and methylmercaptan abundantly from the substances containing sulphur in the urine (C. B. XVI, 701).

(*e*) Decomposition of urea occurs with many cultures, but by no means in all (Barlow, Mann). Compare page 70. Hallé and Dissard, and recently Mann, have demonstrated very minutely the decomposition of urea. Kashida found it so constant that he described the production of ammonia in a lactoso-urea nutrient medium as a characteristic peculiarity as opposed to Bact. typhi (C. B. XXI, 802), while Melchior (Cystitis und Urininfektion, Berlin, 1897) considers the Bact. coli to be the most common cause of cystitis (after previous injury to the bladder), but denies that it can produce ammonia by breaking up urea. Similar negative results were previously obtained by Schnitzler and Krogius.

Distribution.—

(*a*) *Outside the body:* In canal-water, impure water, but also in springs which can scarcely be suspected of pollution, there occur very often organisms which correspond to Bact. coli (v. Freudenreich, Lehmann and Neumann). We never failed to find them in water suspected of containing typhoid bacteria.

The narrower the definition is made, so much the more is the number reduced. Thus, for example, Schardinger (C. B. XVI, 853) declares water organisms, resembling Bact. coli, which ferment grape-sugar and grow in the incubator, to be frequently present, but in spite of it that the Bact. coli is rare. Most of the producers of fermentation are easily differentiated from the Bact. coli by the milk-white, slimy, tenacious growth upon plates (see below).

Regarding the occurrence in dough, compare page 255.

Gordan found it constantly in decomposing fruits (C. B. L. IV, 247).

(*b*) *In the healthy body:* In intestinal canal even in the first milk-stool. It is never absent in any normal human or animal intestine. In the bodies of 32 healthy persons, which were examined from twenty-four to thirty-six hours after death, the B. coli was present 16 times, especially in the liver and kidneys, doubtless having wandered out from the intestine. Wurtz and Hermann (C. B. XII, 388).

(*c*) *In diseased human body* (the motile and non-motile forms are not often separated): As the cause of numerous diseases, especially of the abdominal organs: peritonitis, cystitis[1] (partly alone, especially when the urine is acid, sometimes associated with the Bact. vulgare; see under the latter), urethritis, pyelonephritis, suppurative nephritis, perinephritis. It occurs remarkably often in suppurative strumitis. A number of intestinal affections appear to be associated with virulent forms of the colon group; at all events, according to Dreyfuss (C. B. XVI, 581), the forms isolated from the diseased intestine are much more virulent for rabbits than those isolated from the healthy intestine. Regarding its relation to dysentery, see page 251. Many authors ascribe also certain cases of cholera nostras to it. (Vaughan and Perkins found an organism related to the colon group to be the producer of poison in confectioner's ice. C. B. L. II, 799.) Most cases of "typhoid" or choleriform disease from the eating of diseased meat depend upon it (see below). Axel Host traced the Norwegian disease from the eating of "knetkäse" to infection with the colon bacterium (C. B. XX, 160). More rarely the Bact. coli is the cause of pneumonia (Klein, C. B. V, 625), leptomeningitis of infants, icterus gravis, Winckel's disease (Lubarsch, Virch. Arch., CXXIII), melæna neonatorum, puerperal fever, panophthalmia, infection of wounds (wound-diphtheria). Thoinot and Masselin hold it to be the cause of many cases of myelitis, as they can produce such experimentally in rabbits (C. B. xvi, 919).

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[1] The cystitis microbes, which do not liquefy gelatin, described by different authors (Rebland, Clado, Hallé, Albarran, etc.) under the most various names, appear to be almost always Bact. coli; compare page 247.

(*d*) *In animals:* In septic infections (puerperal fever, septic inflammation of the umbilical cord, etc.) of cattle. Compare hog cholera, page 252.

Experimental Observations Regarding Pathogenic Action.—(*a*) *In animals:* Just like the Micr. pyogenes, the Bact. coli possessed most variable degrees of virulence; the various morphologically and biologically variable characters are entirely useless for determining anything regarding the virulence. According to Valagussa, the virulence of the colon bacteria from the intestine of experimental animals is greater the sicker the animal. In cats vegetable diet produces considerable increase of virulence of the colon bacteria, milk diet a marked attenuation. Subcutaneously the Bact. coli sometimes causes only suppuration, sometimes septicemia; intraperitoneal injection of 1 c.c. of bouillon culture, according to Gabritschewsky, is always fatal for guinea-pigs in about fifty hours. Fifty separately isolated Bact. coli cultures behaved exactly alike in this; bacteria were always present in the heart's blood (C. B. xvii, 833). According to Vallet, cultivation in filtered, sterilized urinal refuse increased the virulence very much (C. B. xiv, 325).

Immunity and Serum Diagnosis.—Active immunization in the usual way is possible. The serum agglutinates coli bacteria. According to many writers (for example, Pfaundler, C. B. xxiii, 9, 71, 131), the agglutinating action of the serum is much greater against the coli culture employed in the immunization than against other cultures, and it is even absent against many other cultures. The new form of serum reaction observed by Pfaundler was only observed in the action of serum upon the culture employed to produce the immunity. It consists in the absence of agglutination and the formation of balls of long threads in twenty-four hours.

(*b*) *In man:* Pathologic etiologic observations, which have the significance of experiments, have been made in man with B. enteritidis Gärtner and B. morbificans bovis Besenau, which are to be considered as examples of the colon bacterium. When ingested in meat, they make men sick. Similar observations have been communicated by Gaffky and Paak regarding meat (sausage), and by Gaffky regarding milk.

Also heated cultures are injurious. Repeated, subcutaneous injections of small quantities, according to Sanarelli, produce an immunity against virulent Bact. coli cultures (not against typhoid). Introduced into the stomach, boiled cultures are less injurious. The gastro-intestinal canal soon becomes accustomed to large quantities of poison, without the occurrence, on this account, of an immunity against the subcutaneous injection of devitalized or living cultures (A. P., 1894, 353).

Special Methods of Demonstration and Culture.—If coli bacteria are abundantly present (stools), the agar plate at 37° is employed for their isolation. After twenty-four hours shake cultures in liquefied 2% grape-sugar agar are prepared from numerous colonies. After sixteen to twenty-four hours all colon bacteria present abundant gas production, which leads to a breaking up of the nutrient medium. (Fig. 11, p. 89.) The varieties which cause fermentation of grape-sugar agar are examined microscopically (to determine whether they are short rods without spores, and whether they are motile) and are transferred to lactose agar, milk, potato, ordinary and grape-sugar bouillon, and peptone water (indol). If few coli bacteria are present (water), then the water concerned has 2% grape-sugar and 1% peptone added and is allowed to stand for twenty-four hours in the incubator, and then plates are prepared. It has also been recommended to add to preliminary cultures 1% to 2% carbolic acid, 0.75% anhydrous soda, and 1% hydrochloric acid, but we have found no advantage from it.

Forms of the Bact. coli described under separate names.

In the scheme for the peritrichous Bact. coli, as we have just described and represented it, there are included very many subvarieties, described as separate species.[1]

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[1] Some investigators—for example, von Stöcklin—undertake to characterize separate forms of coli in relation to the number, length,

We can find no sharp separation between these subvarieties in spite of every effort to do so. Many descriptions are drawn up without any reference as to how the variety being described is related to those next to it, or the differential diagnosis is built upon one or another characteristic whose inconstancy has long since been established either for the colon group itself or even for other exhaustively studied groups (Micrococcus pyogenes, Streptococcus pyogenes, Streptococcus lanceolatus, etc.).

Bacterium coli, var. dysentericum Celli.—Maggiora traced an extensive epidemic of dysentery in northern Italy to the Bact. coli. Arnaud candidly declared the Bact. coli to be the cause of dysentery in hot countries. Celli (C. B. XVII, 309, and XXV, 481) found, as the cause of dysentery in Italy, a form of the Bact. coli which he called Bact. coli dysentericum, and which differs from the Bact. coli only in its pathogenic properties and not in other peculiarities. It grows delicately, more like the Bact. typhi, and ferments grape-sugar and coagulates milk slowly. With this the Bacillus dysenteriæ Shiga (C. B. XXIII, 599, and XXIV, 818) may be considered identical. Both organisms, in distinction to other coli forms, were agglutinated by the serum from cases of dysentery or from animals immunized against this form of Bact. coli. Shiga gives an extensive review of the literature, with illustrations; also a criticism of the works which advocate amebæ as the cause of dysentery. There is still a decided possibility that the clinical picture of dysentery, as especially Kruse and Pasquale suggest, is caused by entirely different agents in separate epidemics. Literature relating to the ameba question is given by Kruse and Pasquale (Z. H. XVI, 1) and Fajardo (C. B. XIX, 753), who consider amebæ to be the cause of tropical dysentery. Ciechanowski and Novak cannot convince themselves of the importance either of amebæ or of forms of the Bact. coli; for many cases certain streptococci appear to them to be primarily responsible (C. B. XXIII, 445). Regarding the questions connected with dysentery, consult also the critical review of the literature by Janowski (C. B. XXI, 234).

Bacillus enteritidis Gärtner.—Morphologically identical, flagella unknown. According to Lubarsch, milk is coagulated, but it was not observed by Günther and Th. Smith. Cause of poisoning by meat; even the broth prepared from the meat was also poisonous. (Korresp. Blätter des ärztlichen Vereins für Thüringen, 1888, No. 9.)

Bacillus of Ferret Plague of Eberth.—Bact. mustelicida Heim. Corresponds, according to our investigations, in all respects to the Bact. coli. It has four or five long, peritrichous flagella (C. B. V, 454, and VI, 87).

Bacterium brassicæ acidæ of Lehmann and Conrad.—Found by Conrad in many samples of sour-crout, and the cause of the fermentation of sour-crout. Has 4 to 10 very long, thin flagella. Often stains slightly by Gram's method. It is differentiated by its production of marsh-gas upon cabbage broth. Besides about 80% CO_2, there is

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and staining properties of the flagella. We should be glad to separate the atrichous (Bact. aërogenes), peritrichous, and mono- and lophotrichous Bact. coli, if it had not been impossible to carry it out.

formed 18% H_2, and 2% marsh-gas. It ferments milk-sugar and coagulates milk (A. H. XXIX, 56).

Bacillus of the Marseilles Swine Plague Jobert and Rietsch. (C. B. IV, 270.)

Bacillus of Spontaneous Rabbit Septicemia Eberth and Mandry (Fortsch. der Med., VIII, 1890, 547).—Milk is coagulated. We do not know regarding the arrangement of the flagella.

Bacillus aphthosus Kruse (Bacillus of mouth and foot disease, according to Siegel; Deut. med. Wochenschr., 1891, No. 49, 1328, and 1894, Nos. 18, 400, and 19, 426; C. B. XIX, 728).—There is no certainty that it has anything to do with mouth and foot disease. According to Kruse, who found the cultures to be motile, it is a typical Bact. coli.

Bacillus indigogenes Alvarez.—In the maceration and boiling of the leaves of the indigo plant, it brings about the formation of a blue pellicle from the pre-existing "glycosid, indican." The bacterium is motile, but otherwise, macroscopically, microscopically, and culturally (capsule, fermentation of sugar, etc.), is very much like the Bact. pneumoniæ Friedländer. The latter is also able to break up indican. The indigo bacillus is also pathogenic (C. B. II, 441). According to recent authors, indigo is formed without aid from bacteria, but by only the combined action of diastatic and oxidizing ferments. (Compare also Bréaudat, C. B. L., Bd. V, 167.)

Bacterium coli β polaris. Lehm. and Neum.

(Plate 18, XII.)

Not distinguishable from the Bact. coli morphologically or biologically except that the flagella are always only at one or both poles. Cultivated by us from cheese ("Emmenthaler") and from the organs of a dead deer; by Stöcklin (C. B. XVI, 130) from feces; cultivated by F. Gärtner from the organs of a dead guinea-pig, and closely studied and found pathogenic for guinea-pigs (C. B. XV, 1).

Lucksch has photographed a similar form as Bact. coli (C. B. XII, 428), only it appears remarkable to us that he comes to the conclusion that the Bact. coli always have 1 to 3 flagella. We, like Stöcklin, have found, among many isolated "coli forms," only a few with a single flagellum, which, so far as we now know, possess this as a constant property. We have not been able to enter into special investigations regarding this.

Bacterium choleræ suum. (Migula.) Lehm. and Neum. (Bacillus suipestifer Kruse.)

Synonyms.—Cause of hog cholera (Salmon), of Svinpest (Bang and Selander, C. B. III, 360; XI, 339; XIII, 203), of the Danish swine plague ("Schweineseuche"), swine plague (Billings), swine fever (Klein, C. B. XVIII, 105). Bacillus choleræ suum Migula. Recently the disease is often spoken of in Germany as "Schweinepest" or "American Schweineseuche."[1]

Principal Literature.—Raccuglia (C. B. VIII, 289); Th. Smith (C. B. IX, 253; XVI, 231); Silberschmidt (A. P. IX, 65); Voges (Z. H. XXIII, 149); Karlinski (Z. H. XXVIII, 373).

This organism is not different morphologically from the Bact. coli. Macroscopically and microscopically (multiple, long, peritrichous flagella), it furnishes a typical form of the Bact. coli.

The following biologic peculiarities, which are confirmed by our study of a culture from Rubner's Institute, serve to differentiate the organims:

1. From milk-sugar it forms neither acid nor gas, and inoculated milk is not coagulated, and does not become acid, but alkaline.
2. The gas produced from grape-sugar is one-third CO_2, two-thirds H_2. (The Bact. coli yielded us similar proportions.) According to Smith, one-half CO_2 and one-half H_2.
3. Does not produce either indol or phenol.

The cultures studied by us were always motile.[2] Ferrier (Lyon Médical, 1894, No. 40) found the hog cholera, after being cultivated for five months upon agar, to present short, very actively motile rods, with multiple flagella, 35 μ to 55 μ long. The micro-organisms 1 μ long had the appearance of spindles. After passage through an animal several times, the rods were longer, the cilia fewer and shorter.

Pathogenic Significance.—The organism causes destructive swine plague in northern countries, such as America; recently also in England, and for about five years in Germany (Graffunder, Deupser, C. B. XVII, 49);

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[1] Voges and Proskauer, in their latest publication (Z. H. XXVIII, 20), designate a form as "schweinepest" which ferments all varieties of sugar, and so corresponds to the type of Bact. coli. However, with the addition of caustic potash to fermenting sugar bouillon, in twenty-four hours, with the admission of air, a red, fluorescent, eosin-like color appears. This color occurs with none of the cultures of American hog cholera, and Voges then also states that in Germany he has so far seen only swine plague ("Schweineseuche"), and no hog cholera. We have found nothing concerning this motile, special "Schweinepest" bacterium in other authors.

[2] Th. Smith has described a non-motile form (without flagella) (C. B. XXV, 241).

recently also in Hungary, Bosnia, etc. Th. Smith (C. B. IX, 253) describes the following forms:

Acute Form.—Hemorrhagic septicemia; hemorrhages especially observed in the lungs, kidneys, and serous membranes (stomach, intestine). Marked splenic tumor. Death in a few days.

Chronic Form.—Animal emaciated, gait tottering. Larger and smaller necrotic areas (ulcers) on lips, palate, tongue. Mucous lining of stomach very red, in places showing ecchymoses. Necrotic areas (sometimes dry, nodular infiltration, sometimes broken-down ulcers) are sometimes seen in the small intestine and rectum, and more often in the cecum and colon. The lungs are not much changed, but there may be some atelectasis or bronchopneumonia. The kidneys are almost always diseased, albumin and casts appearing in the urine. There is a splenic tumor, usually necrosis in the liver. Death in two to four weeks.

Animal Experiments.—Guinea-pigs, rabbits, and pigeons are susceptible.

Differential Diagnosis.—

AMERICAN SWINE-PLAGUE. (Swine-pest = Hog cholera.)	GERMAN SCHWEINESEUCHE. (Löffler and Schütz. See p. 209.)
Bacterium choleræ suum. L. and N.	Bacterium suicida Migula.
Very actively motile.	Non-motile.
Ferments grape-sugar.	Does not ferment grape-sugar.
Luxuriant growth on potato.	Little or no growth on potato.
Rather luxuriant growth on agar, very friable.	Slow growth, on agar, coherent (Karlinski).
No changes at the point of infection.	Marked changes at the point of infection.
Multiple areas of coagulation necrosis in the liver.	Liver often the seat of fatty degeneration.
Few bacteria in blood.	Abundant bacteria in blood of the heart and large vessels.
Very few bacteria at the site of inoculation.	Abundant bacteria in the inflammatory edema at the point of inoculation.
Pigeons very susceptible, guinea-pigs less so.	Guinea-pigs very susceptible, pigeons less so.

The following are closely related to the Bacterium choleræ suum, and differ somewhat more from the Bact. coli on account of the absence of some biologic (not morphologic) peculiarity.

Bacillus of Intestinal Diphtheria Ribbert (Deut. med. Wochenschr., 1887, No. 8, 141).—This peritrichous organism is indistinguishable morphologically from the Bact. coli, yet the culture in our institute (cultivated for eight years upon non-saccharine nutrient media) decomposes grape- and milk-sugar, with intense production of acid, but without gas.

Bacillus diphtheriæ columbarum Löffler.—A culture obtained from Král, which we studied carefully, corresponded exactly, morphologically and biologically, with Bact. choleræ suum: bouillon very cloudy, suggestion of pellicle, milk unaltered, potato at first yellowish then yellowish-gray, finally brown, almost the same as glanders.

Bacterium levans Wolffin (A. H. XXI, 268).—Cause of fermentation in leaven. Many long flagella, milk not coagulated, indol formation overlooked by Wolffin, still it is present after prolonged standing. It also brings about the most varying true coli fermentation of dough (acetic acid, lactic acid; 75% CO_2, 25% H_2) in sterilized flour. More recently we have regularly isolated from sour dough and fermenting bread-dough absolutely typical Bact. coli which at least possess toxic action. Dissertation of Felix Fränkel, Würzburg, 1896.

Bacterium morbificans bovis Basenau (A. H. XX, 241).[1]—Not distinguishable morphologically and biologically from Bact. choleræ suum. It ferments grape-sugar feebly, never coagulates milk, and thus appears not to affect milk-sugar.

Cultivated many times from cattle suffering from a septic disease in which the spleen is enlarged and there are necrotic, whitish-yellow areas in the spleen and liver. The organism is found in the blood, internal organs, and muscles of the diseased animal. Mice, white rats, and guinea-pigs are killed by feeding. Rabbits and the other animals die after infection of the subcutaneous tissue, the peritoneum, or the interior of the puerperal uterus. The organisms escape in the milk. Compare the **Bacterium of Nouvelle septicémie des veaux** of Thomassen (C. B. XXIV, 800).

Compare, further, **Bact enteritidis** Gärtner, page 251, which, as it coagulates milk, is related to the Bact. coli. To one of these two forms appears to belong Gaffky's organism, which, if taken in fresh milk by man, causes severe disease (C. B. XII, 389).

The Swedish **Gaustadt bacillus** of Holst is closely related. Eighty-one persons in the institution for the insane at Gaustadt became sick in 1891, of which four died (C. B. XVII, 717). The disease depended upon the eating of meat. Often there was an initial chill, many times severe backache, sometimes herpes and erythema. The principal symptoms were: fever, vomiting, diarrhea. The organism does not change the reaction of milk. It is motile, having 6 flagella.

Varieties of which nothing is written regarding motility, so far as we know, but which still appear to belong to the Bact. coli (or Bact. lactis aërogenes):

Bacillus aërogenes vesicæ Schow (C. B. XII, 745).

Bacillus of a pigeon plague of Sanfelice (Z. H. XX, 23). Causes sero-purulent peritonitis. Perhaps belongs to Bact. septic. hæmorrhagicæ.

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[1] See there, also, Basenau's investigations, undertaken to establish the difference between his organism and other similar ones.

Bacterium Guillebeau, *a* and *b*, v. Freudenreich. (See C. B. XVII, 487.) The organisms, described in the Annal. de micrographie, which is inaccessible to us, produce simultaneously fermentation of milk (inflation of cheese) and inflammation of the udder.

Bacterium of white or yellow calf dysentery. There is not much to be gained by reference to the works of Piana, Mazzanti e Vigerzi, Monti e Veratti (C. B. XVIII, 653).

Bacterium icteroides. (Sanarelli.) Heim.

Synonyms.—Bacillus icteroides Sanarelli, Bacillus of yellow fever (febris icteroides; Spanish, febre amarilla).

Literature.—Sanarelli (A. P., 1897; C. B. XXII, 181 and 668). The more recent volumes of the C. B. contain many confirmations of Sanarelli's findings by American clinicians and some European authors. Sternberg (C. B. XXII, 145; XXIII, 829; XXIV, 376; XXV, 655).

Regarding the contention whether Sternberg's Bacillus X is the same as the Bacterium icteroides, as Sternberg maintains, we will only say that Sternberg's Bac. X is a typical Bact. coli; the Bact. icteroides resembles more the Bact. typhi.

Sanarelli accepts as the cause of yellow fever a short bacillus, characterized by no special diagnostic peculiarity. It is extraordinarily like the Bact. typhi and many cultures of Bact. coli.

Microscopic.—Short, motile rods with peritrichous flagella, not staining by Gram's method.

In the description of the gelatin [1] and agar culture, nothing is found different from a delicately growing Bact. coli or Bact. typhi. Single peculiarities accentuated by Sanarelli are scarcely constant (compare Agramonte); thus, for example, in agar cultures at room temperature there is shown an elevated ring about a thinner growth (seal culture). The **potato cultures** resemble typhoid, being delicate, colorless, but, according to Agramonte, they become partly brownish later. Milk is not coagulated. In milk-sugar bouillon, no or very little gas is produced. On the contrary, in grape-sugar bouillon it is produced abundantly, but none is formed in cane-sugar bouillon. It is a facultative anaerobe.

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[1] Cultures which we obtained from Král present moderate liquefaction of gelatin and no spontaneous motion, but otherwise corresponded very well to the descriptions given in the text.

Sanarelli rests the principal evidence as to the significance of the organism upon the following:

1. He found it in 58% of cases (the dead bodies).
2. Germ-free filtrate of cultures is claimed to produce in man the entire typical complex of a case of yellow fever.
3. Serum from cases of yellow fever causes agglutination of the Bact. icteroides. Serum from animals which are immunized against the Bact. icteroides is said to operate prophylactically and therapeutically against yellow fever.
4. The B. icteroides is pathogenic for mice, guinea-pigs, rabbits, goats, and sheep. Intravenous injections are followed by vomiting, and bloody enteritis, scanty albuminous urine, and once (!) marked icterus.
5. The pathologic changes in the inoculated animal correspond to those of yellow fever. Often extreme fatty degeneration of the liver occurs. The most convincing preparations are obtained from the dog.

Also Foà (C. B. XXIV, 890) finds grave specific changes in the bone-marrow of animals: fibrinous thrombosis of the peripheral vessels, necrobiotic areas, etc.

We cannot yet look upon these proofs as completely sufficient. While no objection to its pathogenic significance is to be found in the fact that especially sharp peculiarities are not possessed by the Bact. icteroides,—one only has to remember the characterization of the Vibrio choleræ as compared with water vibrios, or the similarity between the Bact. typhi and Bact. coli,—still it is to be admitted that certain varieties of the Bact. coli are also found in many cadavers and may produce similar disease symptoms in animals. It appears also objectionable that yellow fever is a typical disease of the warm zone, and ceases in places and at times with lower temperature, while the B. icteroides possessed about the same resistance as the B. coli to lower temperature. Yet this can also be understood, since the cold may operate upon the intermediate host, carrier, etc.

Bacterium alcaligenes. (Petruschky.) L. and N.

Bacillus fæcalis alcaligenes Petruschky. (C. B. XIX, 187.)

Morphologically very similar to the Bact. coli; luxuriant growth on potato with brown discoloration of the nutrient medium.

No decomposition of any sugar with the liberation of gas; milk is alkaline and not coagulated. Also upon litmus milk alkali is formed. The organism corresponds to the Bact. coli, which has lost its power of decomposing sugar. Differentiation from the Bact. typhi usually not very difficult. Found in the intestine, also in spoiled beer. Compare also Pollak (H. R., 1897, VII, p. 22).

Bacterium Stutzeri. Lehm. and Neum.

Bacillus denitrificans II, Burri and Stutzer (C. B. L. I, 257).

Mention is here deserved by the first completely described bacillus, which, without the aid of synergetic organisms, was able to break up saltpeter, with the liberation of nitrogen. It is a short, motile bacillus (2–4 μ long, $\frac{3}{4}$ μ thick), without spores, and with tapering ends. It grows upon gelatin plates as small, dry, tough, white disks, which are traversed by characteristic radiating ribs, which become united by arches at the edge. The surface growth in the gelatin stab culture is similar; in the stab it grows as a whitish streak. No liquefaction. Agar growth not very characteristic. Upon feebly alkalinized potato, a padded, rib-shaped, thick growth, from a pale flesh-color to peach-red. In bouillon a pellicle forms. In 0.3% nitrate of potassium bouillon there is energetic development of nitrogen. It grows both at room and incubator temperature, equally well without and with oxygen; yet with an abundant supply of air, fermentation of saltpeter is interfered with. The relation to carbohydrates is unknown. Isolated from straw. Found by Künnemann in straw and horse-manure (C. B. L. IV, 906).

Bacterium typhi murium (Löffler). (C. B. XI, 129.) L. and N.

According to Löffler himself, it is very similar in every way, morphologically and biologically, to the Bact. of hog cholera (grape-sugar is converted into acid with accompanying gas-formation). The culture studied by us, like the Bact. typhi, produces acid vigorously from grape-sugar, but no gas; and neither acid nor gas from milk-sugar.[1]

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[1] According to Löffler, feeble acidity is produced in milk, but not sufficient to cause coagulation. Mereshkowski's bacterium from the

Milk remains fluid exactly as with Bact. typhi and is rendered alkaline. Our culture is thus difficult to differentiate from true typhoid, especially since its flagella correspond in number and length with the best flagellated typhoid cultures. On the contrary, the potato growth is remarkably luxuriant.

Upon feeding, the bacterium is pathogenic only for mice; house mice (Mus musculus) and field mice (Arvicola arvalis), but not for Mus agrarius and the various domestic animals. It has been successfully employed to combat the plague of field mice (compare, for example, Zupnik, C. B. XXI, 458, and Appel, C. B. XXV, 373), since the animals, after eating bread soaked in cultures of the bacterium, die; and then, when eaten by their companions, spread the disease still further. The ingestion of 200 germs is certainly, and of 20 almost certainly, fatal (Appel).

Bacillus of mouse plague of Laser (C. B. XI, 184). Almost identical; not studied by us. However, according to Laser, it is stained by Gram's method.

Motile Varieties, Partly Incompletely Described, Related to the Bact. coli or Bact. choleræ suum.

(Statements are lacking regarding the arrangement of flagella or the fermentation of carbohydrates.)

Bacillus of grouse disease of Klein (C. B. VI, 36, 592; VII, 82). Epidemic of the Scotch grouse (Lagopus scoticus).

Bacillus loxiacida Tartakowsky. Cause of crossbill plague. In growth resembles a little more Bact. typhi. No coagulation of milk, no indol.

New gas-producing, aerobic Bacillus of Laser (C. B. XIII, 217). Cause of an epidemic among calves.

Bacterium of an epidemic of young pheasants. Klein (Jour. of Pathol. and Bact., II, 1893, 214).

Bacterium in melæna neonatorum Gärtner (C. B. XV, 865). Typical peritrichous flagella; relation to milk-sugar unknown.

Bacillus pyogenes fœtidus Passet. Untersuchungen über eitrige Phlegmone, Berlin, 1885. Compare also Rabe, Bact. coli as cause of disease in animals (C. B. XXI, 282).

Spermophilus gattatus, a variety of ground squirrel (C. B. XVI, 612, and XX, 176), appears similar to our culture.

Bacterium caniculæ. (Galli-Valerio.) L. and N.

Cause of an epidemic in dogs.

After earlier investigations had given either negative or uncertain results (Microc. pyogenes as cause), recently two authors[1] (Galli-Valerio, C. B. xvii, 677; xix, 694; and Jess, C. B. xxv, 541) claim to have discovered the cause of an important disease of dogs in short, small, motile bacteria (1 to 2 μ long, 0.3 to 0.6 or even 0.9 μ thick). The disease can be successfully transferred to young dogs and cats; according to Jess, also to rabbits and guinea-pigs, but not to mice, the symptoms then corresponding to those of the natural, multiform disease, in which the picture is dominated by fever, ocular and nasal catarrh, protrusio bulbi, and bloody diarrhea.

The statements of both authors do not deviate in any important way.

According to the description of Jess, who needlessly places his findings in contrast with those of Galli-Valerio, the organisms, which are readily cultivated at room temperature, appear to be related to the colon group. They are motile, having a single polar flagellum; gelatin is not liquefied; upon agar there is a gray growth; upon potato a white, velvety growth. In gelatin there occur a few gas bubbles (grape-sugar?). There is a tendency to polar staining by Gram's method. From the description of Galli-Valerio, who places great value upon the form being sometimes shorter and sometimes longer, it appears that old gelatin cultures present funnel-shaped depressions without liquefaction, and that the growth in milk is not accompanied by coagulation or fermentation of milk-sugar. No indol is formed.

Supplement to the Non-liquefying, White, Non-motile, Short Bacteria.

The Bacteria of Acetic Acid Fermentation.

A small group of very closely related varieties form acetic acid from dilute alcohol (for example, beer-wort to which is added 0.5% of alcohol). We have not ourselves studied these varieties, which have so far been studied upon solid media with little thoroughness. Macroscopically the cultures resemble those of the Bact. pneumoniæ, lactici and coli. Thus far, three "species" have been distinguished, **Bacterium aceti** Hansen, **Bacterium Pasteurianum** Hansen, and **Bacterium Kützingianum** Hansen, and they are characterized as follows:

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[1] More extensive reference to the literature is given by these authors.

	Bacterium aceti Hansen.	**Bact. Pasteurianum Hansen.**	**Bact. Kützingianum Hansen.**
Pellicle on sterile ale, at 34° after 24 hours:	Slimy, smooth, moist, shining, showing a tendency to veining like marble.	Dry surface, early beginning to wrinkle, somewhat elevated above the surface.	Similar to Pasteurianum, but the membrane climbs up even on the wall of the tube.
When flasks in which growth has taken place at 34° are brought into room temperature:	Fluid remains clear.	Fluid remains clear.	Fluid becomes cloudy and gradually, under sedimentation, it becomes again clear.
Microscopic character of the cells of the young membrane:	Short rods with hour-glass-like constrictions in chains. Long rods and thread forms uncommon.	Like Bact. aceti.	Short rods, usually single; at most in pairs; no chains.
Staining with iodin of the mucilaginous material holding the bacilli together in young membranes:	Not at all.	Blue. In older membranes the blue staining of the mucus is only presented in places; and in still older, dead cultures, it is entirely absent.	Blue.
Staining of the bacterial cells with iodin:	Yellow.	Yellow.	Yellow.

We gave above a general presentation of the statements of Hansen, the most successful investigator of this group, in tabulated form. Litérature: Lafar (C. B. L. I, p. 129); most important is Hansen, Recherches sur les bactéries acétifiantes (Travaux de Carlsberg, III, 182, and C. B. L. I, 31).

All three forms of acetic acid bacteria possess a wide range of forms, depending especially upon the temperature. This is especially marked in the Bacterium Pasteurianum, according to Hansen.

At temperatures below the optimum of 34°, beautiful chains of short rods are formed; at higher temperatures the short links grow out into long undivided threads. The latter, when again brought to a temperature of 34° or less, in part break up into new short rods, and in part present characteristic bulging. Also the bulging structures are gradually changed, at least partly, under elongation, into short rods, yet the widest parts nevertheless disintegrate. According to Lafar, moreover, the swollen forms are partly dependent upon the action of acid.

During recent years an entire series of new varieties with names have been added to these three old species, which appear to be very difficult to differentiate. (Compare Henneberg, C. B. L. III, 223; IV, 14, 67, 138, and 933.) Also Beijerinck (C. B. L. IV, 209) and his pupil Hoyer (C. B. L. IV, 867) have made surprising new communications regarding acetic acid fungi. Unfortunately, according to the material accessible to us, the description of the morphologic peculiarities leaves much to be desired.

Beijerinck calls Hansen's Bact. aceti, Bacterium rancens Beijerinck, and regards the Bact. Pasteurianum (including Kützingianum) as a variety. Bacterium rancens is the bacterium of sour beer. Here also belong Bact. acetosum Henneberg and Bact. oxydans Henneberg. The true rapidly forming acetic acid bacterium, which Pasteur first isolated, and which Beijerinck now calls Bacterium aceti Pasteur, is entirely different. Hansen, on the contrary, did not know that the Thermobacterium aceti Zeitler belonged here. Finally, Beijerinck distinguished a Bacterium xylinum.

Their recognition may perhaps be accomplished by means of the following key:

(A) Vigorous growth forming a covering in a mixture of tap-water 100, alcohol 3, ammonium phosphate 0.05, potassium chlorid 0.01. Upon beer forms very delicate coverings. Very slight growth upon beer-gelatin, but very luxuriant, slimy growth upon beer gelatin which contains 10 % of cane-sugar. **Bacterium aceti Pasteur-Beijerinck.**

(B) No growth on the above media. Upon beer a vigorous growth forming a covering.

(*a*) Upon gelatin a soft white growth, uninfluenced by cane-sugar.

(*α*) The mucilaginous material secreted remains unstained with iodin. **Bacterium rancens**[1] Beijerinck.

(*β*) The secreted slime is turned blue by iodin. **Bacterium Pasteurianum** Hansen.

(*b*) Upon gelatin, dry, tough, leathery mass; upon beer, there is at first a slimy and then a thick, leathery scum, which gives the reaction for cellulose. Cane-sugar influences the luxuriance of the growth. **Bacterium xylinum** Brown.

How this classification will stand the test of more exact morphologic investigation remains undecided. In the mean time the Bact. xylinum should be considered as a leukonostoc—*i. e.*, it then belongs to the streptococci. Of the other varieties, which are represented as non-motile rods, usually motile forms are also observed. The work of Hoyer is very rich in biologic detail regarding the nutrition of those which produce acetic acid, etc.

Bacterium disciformans. Zopf.

Synonyms.—Bacillus disciformans Zimm. (II, p. 48), Bacillus azureus Zimm. (II, p. 24).

Short rods (0.3–1.4 μ long, 0.3–0.5 μ thick), non-motile, not stained by Gram's method, grows also as an anaerobe. Upon the *gelatin plate:* Very young deep colonies, rather coarsely punctate, round, transparent; superficial colonies, partly like typical young typhoid cultures (especially in the B. azureus), partly somewhat more compact, resembling more the colon type. The superficial colonies liquefy after the second day, when there is frequently seen a narrow hair-like zone. The mass lying on the bottom of the plate is a little denser in the disciformans than in the azureus, and with both there are open spaces. The deep colonies later present little tubercles, and, when they come to the surface, liquefaction and a hair-like rim. In *gelatin stab:* Funnel- to tube-shaped liquefaction, developing rather rapidly. *Bouillon:* very cloudy, abundant H_2S and a little indol produced. Upon *agar:* Dirty white, slimy, luxuriant growth. The agar is colored brownish to rosy red. Upon *potato:* Grayish-yellow to reddish-brown, moderately elevated, moist growth. Grape-sugar is fermented, with abundant formation of gas. Milk is first coagulated, then again rendered fluid.

[1] We found the Bact. rancens not growing very rapidly and somewhat elevated upon solid nutrient media with an oily gloss. No liquefaction of gelatin, no anaerobic growth. Grape-sugar is fermented, with formation of gas and acid.

This variety corresponds, except in the liquefaction of gelatin, to a Bact. lactis aërogenes.

We obtained this variety twice from Zimmermann, once marked Bac. disciformans, the second time Bac. azureus. The varieties corresponded in no way with the description which Zimmermann gave them, but, on the contrary, the two were identical even in the smallest details.

Bacterium punctatum. (Zimm.) Lehm. and Neum.[1]

Synonym.—Bacillus punctatus Zimm. (I, p. 38).

Short rods (0.8 μ long, 0.5 μ thick), often also forming long threads. Actively motile from one polar flagellum. Not stained by Gram's method. Superficial colonies are first roundish, smooth-bordered, transparent, punctate disks; gradually the border becomes finely notched and finally presents beautiful hairy border (somewhat like 41, V). Simultaneously liquefaction begins as a shallow saucer, in which the remnant of the colony can be seen at the center. The border of the saucer is surrounded with a delicate grayish-white zone, sometimes presenting sinuous decorations. The gelatin stab culture at first resembles cholera, but liquefaction rapidly becomes complete. Upon agar and potato the growth is not characteristic, resembling that of the Bact. coli. Milk is coagulated and then the coagulum is liquefied. Grape-sugar is actively fermented, with production of gas. Abundant production of H_2S and little of indol. According to Kruse, this organism, for which he unfortunately suggested the superfluous name **Bac. aquatilis communis**, is one of the most common water bacteria. It corresponds to a Bact. fluorescens without the production of pigment. We have also obtained this organism very frequently from water (if we leave out of consideration the fermentation of sugar, which we rarely tested), and also have often observed forms which were colorless at first for a long time and then became feebly fluorescent.

We found the **Bacillus annulatus** Zimmermann (II, p. 30) very similar in all morphologic and biologic peculiarities; nevertheless it is very well distinguished by habitually producing liquefaction in gelatin in the form of holes. The marked, white accumulation of bacteria which is present beneath the undermined edges of the colonies in the plate culture, the colonies looking as if cut out by a punch, gives a very striking picture.

Bacterium vitulinum. (Weissenberg.) L. and N.

Short rods, motile, not staining by Gram's method, resembling the Bact. coli. Facultative anaerobe. Young gelatin colonies resemble those of B. coli; then there soon occurs a liquefaction of the surrounding medium and the growth breaks up into a crumbly mass. In the gelatin stab there is marked liquefaction, at first funnel-shaped, then cylindric. The content of the funnel is very cloudy, and there is a delicate pellicle on top. Rather marked little hairs, directed downward, which grow out into the solid gelatin about the funnel of liquefaction are quite remarkable.

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[1] An organism, similar in every way, but not forming gas from sugar, was isolated by us from gastric contents.

Bouillon very cloudy, with a delicate pellicle. Abundant H_2S_1 and little indol are produced. Upon agar and potato the growth is nearly the same as that of the B. coli; the potato culture has a very putrid odor. Grape-sugar is fermented, with abundant gas-formation, and milk is not coagulated.

Determined to be the cause of a dysentery in calves in Silesia and given to us by Weissenberg.

The four following are closely related to, perhaps identical with, the described "liquefying varieties of B. coli," and are known to us through the descriptions only:

Bacterium fœtidum liquefaciens. (Tavel.) L. and N.

From one to three short flagella extending out from an unstained capsule (von Stöcklin, Recherches sur la groupe des Coli-Bacillus, 1894).

Gelatin in stab is liquefied, and has a strong fecal odor. Sugar is fermented, with liberation of a vast amount of gas. Milk is not coagulated. Bouillon becomes turbid and a pellicle develops upon the surface.

Bacterium cloacæ. (E. O. Jordan.) Lehm. and Neum.

(Compare Th. Smith, "The Fermentation Tube," 1893, 215.) Surface growth in gelatin is thin, with a somewhat irregular outline. Abundant, uncharacteristic, yellowish-white growth on potato. Actively motile. Very abundant and rapid formation of gas from dextrose and saccharose, the closed end of the fermentation tube containing from 50% to 95% (about one-third H and two-thirds CO_2). Gas is produced more slowly from lactose. Milk is coagulated in eight days.

Bacterium agile (Schou, Flügge). Lehm. and Neum.

Synonym.—Bacillus pneumonicus agilis Flügge.

Cause of the aspiration pneumonia following division of the vagus. Flügge: Mikro-organismen, III. Aufl., page 287, and G. Neumann (C. B. II, 755).

Bacterium pseudomelanosis. P. Ernst (V. A., Bd. 152, p. 418).

In this relationship belongs the interesting organism which Ernst isolated from a case of pseudomelanosis, and recognized as the cause of the same. About the bunches of bacteria there lay in the tissue dark green deposits of sulphid of iron. The organism produces H_2S very actively, forms gas from sugar, liquefies gelatin, has many flagella and no spores, and is not stained by Gram's method.

Bacterium salmonicida. (Emmerich and Weibel.) Lehm. and Neum.

Bacillus of a trout epidemic of Emmerich and Weibel (A. H. XXI).

Non-motile, short rods, more rarely longer rods and threads, not stained by Gram's method. Facultative anaerobe. *Plate cultures in gelatin:* Very young cultures resemble those of the streptococcus; then they sink deep into the gelatin, without real liquefaction, the border of the colony becoming irregular and notched. *Gelatin stab cultures* at first also resemble those of the Streptococcus pyogenes; later (after five to seven days) there occurs a funnel-shaped, steep-walled, deep cavity about the inoculation line, on the sides and at the bottom of which are delicate, whitish bacterial masses. *Agar stab cultures* present flat, moistly shining, irregularly outlined growths of a grayish-yellow color, which after many weeks become brown in the center, and simultaneously the upper part of the agar is discolored brown. *Bouillon* remains clear, only near the top a delicate cloud is formed upon the glass wall, which, upon gentle shaking, sinks very slowly to the bottom as cloudy flakes. A plentiful, whitish sediment gradually collects at the bottom. No growth upon *potato*. No growth at 37°; optimum 10°–15°. We are not acquainted with it.

The organism was cultivated by its discoverers from trout which died in an epidemic in upper Bavaria. Healthy trout were killed by inoculation as well as by adding the organism to water. The principal symptoms of the disease were: at places, where at first there are lentil-sized defects in the scales, furuncle-like swellings gradually develop; then, secondarily, hemorrhagic, suppurating areas form. The organism was abundant in the dead fish, especially in the blood of the heart. The following organism is very similar:

Bacillus devorans Zimmermann (i, p. 48).

Found in well-water. It possesses very active locomotion, but nothing is known of its pathogenic properties.

Bacterium turcosum. (Zimm. ii, p. 32.) Lehm. and Neum.

Very small rods, 0.2–0.3 μ thick and 0.3–1.5 μ long, with sluggish movement, which is due to a polar flagellum.

Upon gelatin plates: small, intense turquoise-yellow, transparent colonies, which gradually sink into the gelatin; microscopically, structureless and more or less transparent. The growth in the gelatin stab culture develops slowly, is smooth, roundish, of an intense yellow passing into a greenish color, and sinks in very slowly, without liquefaction. The agar cultures are similar. Upon potato: scanty, greenish-yellow dry or slightly shining growth. In bouillon there is a little turbidity, without formation of H_2S or indol worth mentioning. Grape-sugar is not perceptibly affected. Milk is not coagulated.

Isolated by Zimmermann from water. In examinations of preputial secretion we have twice obtained cultures which correspond to Zimmermann's original one.

Bacterium cremoides nobis ad interim.[1]

Short rods, 0.5–0.8 μ thick, 0.8–1.6 μ long, non-motile, staining by Gram's method. Gelatin plate: natural size, gray to grayish-yellow disks; magnified 60 times they are finely granular, later opaque and non-liquefying. The gelatin stab is not characteristic; the surface growth gradually becomes thick, whitish, reddish, or cream colored and has an oily luster. In the agar stab the growth has a moist luster and is cream colored. The water of condensation is clear with a pellicle and little sediment. Bouillon is similar. Little indol and H_2S are formed, and no gas from sugar. Milk is not coagulated.

Obtained from the tap-water of Würzburg.

[1] **Bacterium synxanthum** (Ehrenberg) L. and N. *Ordinary name: Bacillus of yellow milk.* According to J. Schröter, it is characterized as follows: Actively motile, short, thin rods, producing a yellow pigment, which is readily soluble in water, but not at all in ether and alcohol. It is decolorized by acids, but the yellow color returns upon treating with alkalis. Milk is colored a bright yellow, the casein is dissolved, and the milk becomes alkaline. The culture which we obtained from Král possessed no motility, clouded the bouillon, and produced a prominent pellicle; coagulated milk, with formation of acid; formed gas from grape-sugar; furnished very luxuriant, moist, yellowish-gray growths, resembling those of the B. coli upon agar and gelatin, without liquefaction, and upon potato developed as a light yellow, much elevated growth with a fatty luster. Stains by Gram's method.

Bacterium erythrogenes. (Grotenfelt.) Lehm. and Neum.

Bacillus lactis erythrogenes Grotenfelt. Bacillus of red milk. *Literature:* Grotenfelt (Fortschritte der Mediz., 1889, VII, 41) and A. Baginsky (C. B. VI, 137).

Non-motile, short rods, 0.8–3.0 μ long and 0.5–1.0 μ thick. Stains by Gram's method. Upon the gelatin plate, grayish-yellow, roundish disks, which gradually sink into the gelatin and liquefy it. When magnified 60 times, at first both the superficial and deep colonies resemble very much those of the Bact. coli; later, when liquefaction begins, the border of the colony, now having become opaque, is beset with fine hairs, and later appears irregularly eaten out and coarsely granular. The intensity of liquefaction is decidedly variable in different colonies. Upon the gelatin stab there develops a sulphur-yellow, thick, slowly sinking growth; later the liquefaction is cylindric. The surface growth upon agar is yellow and moist. Agar and gelatin (especially in the dark) become colored intensely rose-red to garnet. Our cultures also did so in diffuse daylight. According to Grotenfelt, the pigment presents two lines between D and E, and one in the blue portion of the spectrum. Potato culture is sulphur-yellow, elevated, partly dull and partly moist. The cream separates from milk (cream, yellow), the casein forms a flocculent precipitate (with alkaline reaction), the clear serum becoming rose-red. No gas is formed from grape-sugar. From bouillon there is formed abundant indol, but little H_2S. Our description is from a culture obtained from Král.

Bacterium helvolum. (Zimm. I, p. 52.) Lehm. and Neum.

Plump, rather thick, short rods (1.0–3.6 μ long, 0.8–1.2 μ thick), non-motile, staining by Gram's method. Gelatin plate: Colonies are roundish, lively lemon yellow, flatly elevated, and later they sink in the gelatin. When magnified 60 times: homogeneous, hardly at all transparent in the middle, clearer at the edges, border smooth, and with beginning liquefaction the sharp border becomes slightly crumbly.

Upon the gelatin stab culture a luxuriant, shining, intense lemon-yellow growth, which slowly sinks into the medium. Agar culture: yellowish-gray, moist. Potato culture: dull, broad, greenish-yellow. Bouillon becomes cloudy, with a delicate pellicle. Abundant formation of H_2S, but none of indol. No gas is formed from grape-sugar. Milk is coagulated.

We obtained an organism from air which corresponded exactly with Zimmermann's description. **Bacillus luteus** Flügge appears identical, except that liquefaction is absent. Also the following appear very closely related: the non-liquefying **Bac. constrictus** Zimmermann (I, p. 42) and the **Bac. subflavus** Zimmermann (I, p. 62).

Bacterium lactis saponacei. (Weigm. and Zirn.) Lehm. and Neum.

As the Bacillus lactis saponacei, Weigmann and Zirn (C. B. xv, 463) have described a short rod, which in gelatin plates forms white colonies with yellow centers, which later become yellow throughout, but without special markings. Gradually liquefaction takes place. In the gelatin stab a funnel forms, at the bottom of which lie yellow flocculi. In the agar stab the luxuriant growth is yellow in the center only at first, then throughout the whole growth. Upon potato a waxy-yellow, slimy growth. Milk is not coagulated, but becomes slimy and slightly tenacious. The culture has an odor like soap or lye. Optimum at 10°. Regarding soapy milk, the first communication was by Herz, Ch. Zeit. Rep., 1892, page 34.

Bacterium nubilum. (P. and C. Frankland. Z. H. vi, p. 386.) Lehm. and Neum.

Non-motile short rods, 1-2 μ long, 0.3-0.5 μ thick, staining by Gram's method. The colonies on the gelatin plate present beautiful, polymorphous forms. In the younger stage they are yellowish, of irregular forms, and provided with many thick and thin lateral outgrowths, similar to mites in shape. The more compact nucleus at the center gradually disappears, while the projections become arranged more in the form of a star. Now liquefaction of the gelatin begins. The periphery of the colony slowly dissolves into delicate little fragments, and in the fluid contents of the saucer of liquefaction there remains a framework of radiating threads, which later become arranged like the spokes of a wheel. Finally the entire colony breaks up into irregular fragments. Macroscopically the colony does not appear unlike that of the Bac. subtilis. In the gelatin stab the growth sinks in, with the form of a saucer, and then cylindric liquefaction occurs. The liquefied zone is slightly cloudy. The growth upon agar is jagged, undulating, fairly luxuriant; in the center, pale rose color; at the edges, yellowish-brown, with a fatty luster. The water of condensation is clear with a yellowish-brown sediment. The growth upon potato is at first entirely reddish-white, faintly shining to dry; later it becomes intensely brownish-yellow. Milk is not coagulated, and is alkaline in reaction. No gas is formed from grape-sugar. It forms but little indol. Bouillon becomes cloudy. Isolated by Zimmermann from water (I, p. 28). Our description is from one of Zimmermann's cultures.

Bacterium ochraceum. (Zimmermann, i, p. 60.) Lehm. and Neum.

Short rods, 0.5–0.8 μ thick, 1.2–3.6 μ long, actively motile from polar flagella, staining by Gram's method. Gelatin plates at first present forms like those of the Bact. coli and typhi; later the borders are fringed, while the gelatin becomes liquefied. Pellicles varying in color from gray to grayish-yellow float upon the liquefied medium, and they may be of a tougher or more delicate character. The more delicate pellicles often appear as a net with irregular meshes. The gelatin stab culture presents a yellowish-gray surface growth, but it sinks in at once. Later there is a cylindric, turbid liquefaction, with grayish-yellow sediment. The agar growth is dirty, light grayish-yellow, then spreads out. The water of condensation is clear, with moderate precipitate. Bouillon becomes lightly cloudy, with moderate sediment and slight pellicle. Indol and H_2S are formed in abundance. Milk is not coagulated, and becomes somewhat slimy. No gas is formed from grape-sugar. The growth on potato is yellowish.

This organism, isolated by us from gastric contents, corresponds in all the main points with Zimmermann's description. We isolated a very similar but non-motile bacillus from Secale cornutum. From this we cannot distinguish a **Bacillus plicatus** Zimm. (I, p. 54), which we obtained from Zimmermann, but it did not form folds any more. Also **Bacterium carnosum** (Tils, Zimmermann, II, p. 4) is very closely related. We were unable to find the spores, seen by Tils; also, the color of the culture obtained from Zimmermann could not be distinguished from that of the Bact. ochraceum.

Bacterium fulvum. (Zimmermann.) L. and N.

Rods, 0.3–0.5 μ thick, with a length varying from 1.0 μ to long threads. Non-motile, without flagella, staining by Gram's method, sometimes liquefying, sometimes not.

Gelatin plates: Shining, orange-yellow colonies, sometimes more drop-like, sometimes more spreading, with moderate or no liquefaction. The non-liquefying, superficial colonies, when magnified 60 times, are at first very much like those of Bact. coli; they are irregularly roundish to leaf-shaped, somewhat transparent, grayish-yellow, homogeneous, often having furrows and markings resembling the Bact. coli. The liquefying colonies present an essentially different appearance: The yellow, superficial disks have a threaded border resembling subtilis (compare 40, II); later the colonies break up into a crumbly mass which lies at the bottom of the liquid.

In the *gelatin stab* there is no striking growth. The surface growth is leather-brown to orange and reddish-orange. When liquefaction occurs, there is formed a funnel, filled with turbid fluid; later the liquefaction became cylindric and sometimes there is a pellicle.

Agar stab: Succulent orange-yellow to yellowish brownish-red. (Compare, for example, 5, v.) *Potato growth* is the same.

Milk: It is not coagulated, but both of our liquefying forms changed it into a yellowish turbid fluid, with an orange sediment, upon which the yellowish cream floated. A non-liquefying form coagulated milk (original culture of Bact. tremelloides Schottelius). No gas is formed from sugar. Little indol and no H_2S are produced. Found by us in water and milk.

We consider that the following varieties, which we have ourselves investigated, belong here: **Bacterium bruneum** Schröter, which we obtained from A. Fischer; **Bacterium tremelloides** Schottelius, obtained from the discoverer himself. The description of Zimmermann's **Bacillus fuscus** Flügge corresponds completely.[1]

The **Bacterium mycoides roseum** Scholl appears very closely related, although deviating somewhat in color (Fort. d. Med., VII, 46).

What we obtained from Hauser as **Bacillus arborescens** Frankland is also the same, and neither corresponds with Frankland's original description (Z. H. VI, 379) nor with that of Zimmermann. The deviation from Frankland consists in the loss of liquefaction (absence of bundles); in Zimmermann's description it is said not to stain by Gram's method. We have never been certainly convinced regarding motility, and so far have been unable to stain flagella.

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[1] The description given by Schröter himself of his **Bact. bruneum** corresponds very poorly, as does the description of Flügge of his Bacillus fuscus. Therefore we select the oldest of the newer names, which is characteristic, and the description of which corresponds well with our cultures.

Bacterium chrysoglœa. Zopf.[1]

According to Zimmermann's description (II, p. 12), it is only distinguished from the preceding by active motility. We found in gastric contents an exactly corresponding form with peritrichous flagella and active motility, which stained by Gram's method. Chrysoglœa and fulvum may be related, as Forma mobilis and immobilis. Proof is still wanting.

Bacterium latericium. (Adametz.) Lehm. and Neum.

(Plate 20, I–VI.)

Short rods, somewhat pointed at both ends (0.8–1.6 μ long, 0.4–0.6 μ thick), non-motile, and stained by Gram's method. Upon the gelatin plate the deep colonies appear as roundish, reddish-brown, opaque disks with smooth edges. The deep ones are jagged, sinuous, transparent at the edge, very crumbly, and reddish (20, III). In the gelatin stab no liquefaction occurs, the surface growth is from vermilion to reddish-brown (20, II). The growth upon the agar streak is the same (20, I). The growth upon the agar plate is not especially characteristic; round disks, coarsely crumbly, border granular, and in the deep ones smooth (20, V). Upon potato the bacterium grows very slowly only and very scantily (20, IV). Bouillon remains clear. Milk is not coagulated. Neither gas nor acid is formed from sugar. No H_2S, and only traces of indol are formed. Isolated by us from the air; corresponds, so far as can be judged from Eisenberg, with the description of Adametz. The organism does not belong here, according to its atural relationship, but more properly with the Bact. acidi lactici.

Catiano has described two other bacilli, which are motile, beauti- ly provided with flagella, produce red pigment, and do not possess res: Bac. rubiginosus and coccineus (Cohn's Beitr., Bd. VII, 6, H. III, 537). We could not study these.

acterium prodigiosum. (Ehrenberg.) Lehm. and Neum.

(Plates 21 and 22.[2])

Synonyms.—Monas prodigiosa Ehrenberg, Micrococ- us prodigiosus Cohn, Bacillus prodigiosus Flügge.

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[1] Migula places **Bact. chrysoglœa** with the non-motile varieties, d designates **Bact. aureum** Frankland, **Bact. aurescens** Frank- d, and **Bact. egregium** Zopf as closely related.

[2] The plate drawn for Bact. kiliense has forms which also occur

Most Important Literature.—Schottelius (C. B. ii, 439); Wasserzug (A. P., 1888); Kübler (C. B. v, 383); Scheurlen (A. H. xxvi, 1).

Microscopic Appearance.—From solid nutrient media, very short bacilli, often looking like cocci. The ends are somewhat pointed or rounded. The greatest diameter is 1μ (21, xi; 22, ix). In bouillon, especially if it is faintly acid, there occur longer forms, distinct rods, and shorter and longer threads.

Motility.—In young bouillon cultures there is active motion, produced by from 6 to 8 long, peritrichous flagella (21, xii; 22, xi). On the contrary, older agar and potato cultures appear non-motile, and in them the bacillus produces abundant slimy material, which limits motion. Scheurlen attributes the mucous formation to the abundant production of alkali.

Staining Properties. — Easily stained, but not by Gram's method.

Relation to Oxygen. — Facultative anaerobe; grows better as an aerobe. Also as an anaerobe it liquefies gelatin (also with the addition of 2 per cent. sugar), but forms no pigment.

Requirements as Regards Temperature and Composition of Nutrient Media.—Optimum at 22°–25°; in the incubator, especially at 38°–39°, the formation of pigment is suspended. A more prolonged cultivation at a higher temperature permanently lessens the formation of pigment.[1] It grows also, with production of pigment, upon non-albuminous nutrient media.

Gelatin Plate.—(*a*) *Natural size:* At first the superficial colony is a grayish-white point, and the gelatin is liquefied at once. The area of liquefaction is shaped like a plate. The peripheral zone is lighter than the central zone. Original colonies are often colored reddish, but often

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with the Bact. prodigiosum, since both are identical (compare p. 276).

[1] It may be here remarked that, without known cause, chromogenesis by the Bact. prodigiosum is often much reduced. As is often seen, of 20 cultures made at the same time and from the same originals upon the same nutrient media, many form pigment abundantly and others very feebly. Also, upon plates fainter and more deeply colored colonies always occur side by side.

they remain white and disappear with the increasing size of the area of liquefaction. Thus the paler zone disappears, and the entire liquefied area becomes colored uniformly gray (21, VIII; 22, III).

(*b*) *Magnified seventy times:* Superficial colonies, at first delicate, granular, roundish, with a smooth border; later the central zone is colored rosy red, is delicately crumbly, and sometimes has a faint suggestion of streaking. The peripheral zone consists of continuous little tufts of hairs, which terminate externally in very fine points (21, VII; 22, IV). Besides this form, there are often atypical ones with a brownish center, the separate zones being lost, and the whole colony appearing covered with extremely delicate hairs. One form passes into the other. The deep colonies are uncharacteristic, yellowish-brown, granular, whetstone-shaped.

Gelatin Stab.—After six hours liquefaction begins at the surface of the gelatin in a saucer shape. The liquefaction extends along the stab canal, forms a tube- or cone-shaped funnel, and continues to possess a funnel form in the advanced stage. Only after a very long time does the liquefaction become cylindric. The funnel of liquefaction is filled with whitish or rose-red flocculi, among which more deeply stained clumps are swimming. When liquefaction has advanced very far, a cloudy, reddish to deep red precipitate is at the bottom and the supernatant fluid remains red. When the culture grows atypically, no red color is seen. The form of the funnel of liquefaction is most variable (21, I; 22, II).

Agar Plate.—(*a*) *Natural size:* The colonies appear as minute red points even after thirty-six hours. Those lying upon the surface increase in size perceptibly and become colored from rose-red to dark red. Also, uncolored colonies occur together with these. They are irregularly roundish, sometimes lobed, often with alternating paler and darker zones and distinct cloudy center (21, V; 22, VI).

(*b*) *Magnified seventy times:* Both the deep and superficial colonies at first are roundish, of irregular form, pale yellow with smooth border. Later the deep colonies take on a brownish color with a reddish luster, the border remaining smooth and the structure coarsely granular. On the contrary, the superficial colonies are transparent, pale rose-red to red, very finely punctated, with borders almost or entirely smooth (21, VI; 22, VII).

Agar Stab.—*Stab:* Thread-like, without nodules, white to reddish. After keeping longer, a whitish cloudy zone forms about the stab canal (21, III). *Surface growth:* Already after forty-eight hours completely covered with a smooth, shining growth, the color of which varies from atypical white to typical purple (21, IV). Often it is whitish-gray, shaded with red. The agar, especially beneath the surface growth, after a longer time becomes colored a garnet-red.

Agar Streak.—The growth remains limited to the streak; compare agar stab. The water of condensation presents a reddish cloud with a red sediment (21, II; 22, I).

Bouillon Culture.—Diffuse, marked turbidity, with a more or less red-colored, delicate pellicle upon the surface. The bouillon becomes of a gelatinous or oily consistency.

Milk Culture.—After twenty-four hours it is firmly coagulated; later the coagulum is dissolved and a yellowish color produced.

Potato Culture.—At first a rosy red, moist, flat growth, limited to the inoculation streak. Later it becomes darker in color, is elevated, with a wavy, smooth border, and after five or six days has attained its dark purple color (21, IX; 22, X). Sometimes the surface then exhibits a greenish-golden reflex, similar to dry fuchsin. Also the potato culture develops atypically at times, as does that upon agar, and becomes only whitish-gray, orange, or rose-red, instead of dark red (21, X).

Chemical Activities.—

(*a*) The red pigment (prodigiosin): Develops best upon agar and potato, is insoluble in water, and only externally in color and golden luster is it like fuchsin; according to Scheurlen, it is apparently also free from nitrogen besides containing no sulphur nor phosphorus. The pigment is readily soluble in alcohol and ether, is turned orange-yellow by alkalis, and from carmine to violet-red by acids. With zinc and hydrochloric acid the pigment, notwithstanding contrary statements, is decolorized, as are all red pigments of this group. In light it fades rapidly as well when dry as when in solution. The pigment, spectroscopically, is sharply characterized; more detailed communications thereon will soon follow from this laboratory.

(*b*) Olfactory and gustable materials: Especially upon potato it forms methylamin and ammonia. According to Schottelius, the odor is proportional to the pigment production, but we found also colorless cultures with a marked odor, as of herring.

(*c*) Production of gas and acid from grape-sugar: Fairly active, according to Schottelius and other authors; on the contrary, our prodigiosum culture formed acid without gas (but our kiliense formed gas). A prodigiosum isolated by Cramer from the tap-water of Heidelberg also formed no gas. Scheurlen demonstrated the production of formic and succinic acids.

(*d*) Urea is converted into carbonate of ammonia, but not by all cultures.

(*e*) Traces of indol, no H_2S.

Distribution.—Upon cooked potatoes, moist bread, paste, especially upon starchy substances, occurring epidemically often, especially in the late summer and autumn. (Compare Scheurlen.) Cause of the "bleeding host." Sometimes found in water-pipes.

Pathogenic Significance.—If injected alone, is not pathogenic, but may be when combined with other bacteria. The proteins of the prodigiosum have been studied many times and found to be poisonous.

Varieties Identical with or Closely Related to the Bact. prodigiosum.

Bacterium kiliense. (Fischer and Breunig.) L. and N.

(Plate 22.)

Compare Kieler Wasserbacillus, Breunig, Dissertation, Kiel, 1888. Laurent (A. P., 1890, 465; C. B. IX, 105).

The culture which we used for preparing illustrations (Plate 22) is distinguished from the Bact. prodigiosum (Plate 21) by more of a brick-red or orange-red color. This, however, according to our more recent observations, is not constant; prodigiosum may grow with orange, and kiliense with bluish-red color. The formation of alkali is most important as to the color: with abundant production of alkali it is yellowish-red; in other cases, bluish-red. We also found to be absolutely identical **Bacterium miniaceum**[1] (Zimmermann, L. and N.) and the **Bacterium indicum** (Koch, L. and N.), isolated by Koch from an Indian monkey, of which we obtained beautiful red cultures from Král and carefully studied them.

It is very probable that these are also identical:

Bacterium of red pus Ferchmin (C. B. XIII, 103), which differs in being non-motile and staining by Gram's method.

Red water-bacillus Lustig (C. B. VIII, 33).

Bacterium plymuthicum Fischer. (L. and N.) Compare Voges (C. B. XIV, 301).

Bacillus fuchsinus Boekhout and Otto de Vries (C. B. L. IV, 497).

The following is, at any rate, closely related.

Bacterium piscatorum. Lehm. and Neum.

Microbe rouge de la sardine of the French. Causes, in combination with an anaerobic bacillus, panaritium in fishermen, apparently originating in spoiled bait. In boxes of sardines it causes a red color (Du Bois Saint Severin, A. P., 1894, 152). The pigment is soluble in water (?), usually poorly developed upon agar, and is produced at 37°–39°. More extensive studies are required to establish the constancy of these characteristics.

Bacterium violaceum. (J. Schröter.) L. and N.[2]

(Plate 23.)

Synonym.—Compare page 279. Bact. janthinum Zopf. Schröter's name is older.

Microscopic Appearance.—Thin rods, 1.6–5 μ long, 0.5–0.8 μ thick, with rounded ends; the smallest are often oval; sometimes threads form. In the interior unstained areas sometimes remind one of chicken cholera.

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[1] What we obtained from Král as **Bac. rosaceus metalloides** Dowdeswell is entirely different. We have called this **Bact. rosaceum,** and found it to be a fine, small, motile rod, which something grows like the Bact. coli on ordinary media, but with a brick-red color. The pigment is not prodigiosin. Milk and bouillon present brick-red pellicles. No gas is formed from grape-sugar. Milk is not coagulated. Not stained by Gram's method.

[2] Twice in cultures, according to Migula's method, upon quince-juice we have seen pictures which may have been spores.

Motility.—Active, serpentine motion. We found the flagella to be sometimes peritrichous (3–4, long, tortuous), sometimes polar (1–2) (23, XI and XII).

Staining Properties.—Stains by Gram's method.

Growth is moderately rapid, and best at ordinary temperature.

Gelatin Plate.—*Natural size:* At first, small, yellow points, later violet. If the liquefaction is rapid, then there is a gray saucer-shaped depression with violet, alternating concentric rings (23, VII). Where colonies do not liquefy, or do so late, they appear as lobulated, fringed, shining, yellowish to violet growths (compare 23, VIII). *Magnified sixty times:* In both weakly and actively liquefying colonies, they almost always at first resemble those of the typhoid. When sunken in, the colonies become crumbly, have a streaked peripheral zone consisting of little hairs, and finally disintegrate into crumbly masses (23, VIII). Colonies which liquefy very late are internally of a darker, yellow, finally bluish color and opaque, with a crumbly structure.

Gelatin Stab.—In freshly isolated varieties the liquefaction after two or three days is funnel-shaped; and along the stab canal, tube-shaped. The contents of the funnel are grayish-violet with colored fragments (23, I). After longer cultivation (as in our culture, after two years) liquefaction is almost entirely lost. The surface growth now is shining, lobulated, dirty yellow to violet. Only after two to three months is there a very shallow saucer-shaped depression.

Agar Culture.—Moist, shining, somewhat elevated, of the same color as the colonies upon gelatin. In the plate, when slightly magnified, the colonies resemble those of the Bact. coli, and are yellowish-gray and faintly granular (23, V).

Potato Culture.—Wavy, somewhat elevated growth, moist, shining, violet to violet-black. We have also observed, in numerous potato cultures, dirty yellow to brownish-green growths, resembling those of Bact. coli and fluorescens (23, X).

Bouillon.—Faintly or strongly turbid, sometimes provided with a thick, sometimes with a delicate pellicle. In favorable cases the pellicle may assume a pale violet color.

Milk.—In some cases it is coagulated, but it usually remains fluid and is violet in color, at least forms a violet cream layer.

Chemical Activities.—In grape-sugar bouillon there is formed little acid and no gas. It produces abundant H_2S and a moderate amount of indol.

Regarding the pigment (janthin), see page 67.

From the one just described we are unable to distinguish, by any peculiarities worth mentioning, the **Bacterium janthinum** Zopf (Sweden and America), obtained from Zimmermann, and a similarly named bacterium from Král, and a bacterium isolated during the summer of 1894 from the well of the local fort.

A beautiful chromogenic culture obtained in 1898 from Hohnl (Prague) corresponds entirely with the description except that the liquefaction was prominently punched-out in appearance and it did not stain by Gram's method. Also, it seems to us, from a study of the literature, that it is scarcely possible to differentiate a **Bacillus violaceus** Laurentius (Lustig, p. 103), cultivated from the water of a filter basin of Lawrence, a **Bacillus violaceus** Macé (Ann. d'hygiène, 1887), and the **Bacillus violaceus** (Lustig, p. 75), from tap-water of Berlin and London. The latter, according to Voges, is identical with the **Bacillus lividus** of Plagge and Proskauer (Z. H. II, 463), except that the latter is differentiated from the violaceum by growing less well upon potato and by rapid liquefaction. All these characteristics, as follows from what has already been said, are not sufficient for determining a separation of species. Also closely related is the **Bacillus membranaceus amethystinus** (Eisenberg, 1891, 421), cultivated by Jolles from well-water. It produces large violet pellicles upon gelatin and is non-motile. Germano likewise cultivated a membrane-forming organism (C. B. XII, 516), which he named the **Bacillus membranaceus amethystinus mobilis.** It agrees with the preceding except in being motile. Also here it is probable that two identical varieties are found, the one motile, the other non-motile. This is in accord with Ward's discovery of an organism which belongs here, and which sometimes was motile and sometimes not (C. B. L. IV, 902).

Bacterium indigonaceum. (Claessen, Schneider.) L. and N.

Obtained from Král, from Prague. Rods, 1.6–3 μ long, 0.8–0.9 μ thick, somewhat thicker than violaceum, sometimes curved. Upon the gelatin plate, which is not liquefied, there appear, macroscopically, small, blue, drop-like growths. When slightly magnified, they are sharply rounded, yellowish disks, slightly granular and later becoming indigo-blue from the center outward. Upon the agar plate they are similar. The surface growth in the gelatin stab is sky-blue, moist, sometimes also remaining white. The growth upon potato is deep indigo-blue, somewhat granular; later it presents a coppery-red, metallic luster, very similar to solid indigo. It renders bouillon cloudy and forms a pellicle upon it. Milk is not coagulated, but is colored bluish-green. The bacterium is not motile. We have not examined it for flagella. Regarding the pigment, see page 67.

The original description of Claessen (C. B. VII, 13) and the description of Voges of the **Bacillus indigoferus,** which was obtained from tap-water in Kiel, differ only in the statement that the latter organism is actively motile, and that this depends upon a polar flagellum. We examined a culture from Král and verified all the statements of Voges, so that here also are two varieties which differ only as regards flagella, and which really belong together.

Bacterium cæruleum. Voges. (L. and N.)

Literature.—Voges (C. B. XIV, 301). Our description is from a culture from Král.

Microscopically, longer and shorter motile bacilli, resembling the Bact. coli. Do not stain by Gram's method.

They grow well also anaerobically.

Gelatin stab: surface growth thin, with a dull luster; deep blue, slowly becoming depressed. Stab, thread-like, h little nodules. The surface growth in agar has a moist luster, is scarcely at all elevated, with a gray zone at the periphery, and a sky-blue one at the center, and with some diffusion of the pigment into the agar. Upon bouillon there is a thick, tough, somewhat wrinkled, deep blue pellicle, the bouillon becoming moderately turbid. Milk is unchanged, the surface light blue. Upon potato a layer is formed, which is light blue at first, and later becomes dark blue to dark blackish-green. Potato becomes grayish-green throughout. No gas is formed from grape-sugar. We found a trace of the pigment soluble in glacial acetic acid, but it is entirely insoluble in all ordinary solvents.

Bacterium pyocyaneum. (Gessard, Flügge.) L. and N.

(Plate 24.[1])

Synonyms.[2]—Bacillus pyocyaneus Flügge, Pseudomonas pyocyanea Migula, Bacillus of greenish-blue pus, "green or blue pus."

Literature to 1893 by Jakowski (Z. H. XVI, 475).

Microscopic Appearance.—Slender rods, often growing into threads. Thickness, 0.4 μ; length, 1.4 to 6 μ. Other authors have also observed transition forms, from slender rods to short, plump, even almost round forms (24, IX).

Motility.—Actively motile by means of a polar flagellum (24, X).

Stains with anilin dyes and by Gram's method.

Requirements as Regards Nutrient Media, Temperature, and Oxygen.—Usually is a strict aerobe, but is also cultivated from closed abscess cavities. Jakowski (Z. H. XV, 474) has cultivated from an intestinal fistula a form growing anaerobically and in carbonic acid. It is not very particular as to nutrient media and grows rapidly at room and incubator temperature.

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[1] Our plate is painted from a culture which was not entirely typical, as it only forms a little pyocyanin. The color may be much more bluish-green.

[2] See page 285, *et seq.*, for related forms.

Gelatin Plate.—(*a*) *Natural size. Deep:* Roundish to whetstone-shaped, yellowish-white to greenish-yellow. Sometimes also there is a roundish, spreading, transparent, greenish-yellow extension with the original colony in the center. *Superficial:* At first roundish, uneven, delicately spreading, but immediately saucer-shaped liquefaction occurs. There is often a lighter peripheral zone. The liquefied material is cloudy, and gray to greenish-gray. The original colony appears as a crumbly mass at the center (24, V). There is intense fluorescence about the colony.

(*b*) *Magnified fifty times:* Both the superficial and deep colonies are yellowish, roundish, with smooth border, and delicately punctate at first. After twelve to twenty-four hours the superficial colonies have a transparent, ragged border (like Bact. coli), and are also sometimes beset with little hairs or fringes. Then immediately begins the depression of the colony (24, III). The color becomes brownish, the irregular form and ring of hairs are partly lost, the contents of the liquefied area are uniformly crumbly. The periphery and the structure of the colony appear with the greatest variations, sometimes ragged, sometimes granular, sometimes punctate, sometimes lighter, sometimes darker, until the colony falls entirely apart. The middle portion of the colony usually survives and is darker in color (24, IV). (Compare also 25, V and X.)

Gelatin Stab.—Liquefaction begins very early, is at first cup-shaped, later cylindric, and more rarely is shaped like a pointed funnel. The liquefied material is slightly cloudy, with a greenish-yellow to bluish-green fluorescence. There is gradual liquefaction along the stab canal, the contents being yellowish and crumbly (24, I).

Agar Plate.—(*a*) *Natural size. Deep:* Roundish to whetstone-shaped, non-characteristic, yellowish. *Superficial:* Roundish, smooth-bordered, with a moist luster, greenish-white to yellowish. There is intense greenish-yellow fluorescence of the surrounding medium (24, VI).

(*b*) *Magnified fifty times. Deep:* Roundish to whetstone-shaped, with a border partly smooth, partly delicately wavy, delicately punctate or granular (like Bact. coli), light yellow to greenish-yellow. *Superficial:* Usually round disks, with border almost smooth, more or less strongly granular, very often also moruloid, light yellow to greenish-yellow. Except for the color, it is not distinguishable from Bact. fluorescens, putidum, and coli (24, VII). (Compare also 25, VI; 26, VIII.)

Agar Stab.—*Stab:* Non-characteristic, thread-like, and a little nodular. *Surface growth:* Whitish-gray to greenish, dull to moistly shining. In forty-eight hours it is uniformly spread over the entire surface. The agar has a yellowish-green to bluish-green fluorescence.

Agar Streak.—Somewhat spreading growth, with a moist luster, wavy, smooth border, yellowish-green in color. The agar shows marked blue to yellowish-green fluorescence. The water of condensation is almost clear; there is a white precipitate and a whitish pellicle on the surface (24, II).

Bouillon Culture.—Marked yellowish-green fluorescence. Very turbid. Moderate quantity of sediment, which is broken up with difficulty upon shaking. Pellicle upon the surface.

Milk Culture.—Milk is coagulated, and later again liquefied. The liquefied portion presents yellowish-green fluorescence. Reaction is always alkaline.

Potato Culture.—At first a yellowish growth, with a moist luster, wavy irregular border, and but slightly elevated; later, brownish-yellow to brown or reddish-brown. Often there is a fluorescent zone about the growth (24, VIII). According to the character of the potato, there is very great variation in the luxuriance, fluorescence, and color, and so the growth cannot be distinguished at any time with certainty from that of other fluorescent varieties. (See also 25, IX.)

Sensitiveness to Injurious Agencies.—Drying kills rapidly. The action of the sun's rays for four hours does not entirely suspend chromogenesis.

Chemical Activities.—

(*a*) *Chromogenesis:* In its typical cultures the Bact. pyocyaneum forms two pigments: a green-yellow, fluorescent bacteriofluorescein, soluble in water, and the beautiful blue, crystalline pyocyanin, soluble in chloroform (see p. 68). There are cultures, however,—like the one represented in our plate,—which produce scarcely any pyocyanin, only much bacteriofluorescein. We have often seen cultures which form upon wafers abundant pyocyanin, which can be easily extracted with chloroform from nutrient media containing water. There are also cultures which, at least on certain nutrient media (it is recommended to employ 1% peptone, 1.5% agar boiled in water, and, finally, 5% gelatin added), produce only pyocyanin, and, finally, there are those which produce no pigment. The brown color of old cultures comes from a changing of pyocyanin into a reddish-brown pigment. Pyocyanin is easily changed into yellow pyoxanthose. Regarding pyocyanin, see also Borland, C. B. xxv, 897.

Regarding interference with the formation of pigment brought about by other bacteria (for example, Micr. pyogenes, Bac. anthracis), see Mühsam and Schimmelbusch (C. B. xv, 430).

(*b*) *Other products:* Upon all nutrient media there is present at first a delicate aromatic odor (compared to linden blossoms). We have also often perceived this odor in other cases; for example, in Sarcina lutea, Micrococcus luteus. Old cultures smell disagreeably of ammonia. It forms neither indol nor H_2S, and from grape-sugar little acid and no gas are produced. Even the boiled bouillon cultures are strongly poisonous. They contain, besides proteins, toxic metabolic products. Nitrogen is liberated from nitrates and nitrites (Lehm. and Neum.). Weissenberg has, in our institute, demonstrated this property in all the four cultures of B. pyocyaneum examined (A. H. xxx, 274).

Experimental Observations with Animals.—It is usually weakly pathogenic for animals; when injected, it causes suppuration. Schürmayer found in mice, after subcutaneous injection, clear edema and serous exudate into the body cavities. Virulent cultures kill guinea-pigs when injected subcutaneously and intraperitoneally.

Immunity.—The very interesting studies of Wassermann (Z. H. xxii, 263) are mentioned on page 110. For more details the original must be consulted.

Distribution.—

(*a*) *Outside the body:* So far, has not been certainly found.

(*b*) *In healthy body:* Sometimes in the mouth and intestine and upon the skin of healthy persons.

(*c*) *In diseased body:* Not infrequently (especially formerly) in pus from open wounds, also in the dressings from wounds, sometimes in epidemics in the rooms of the sick. Usually the organism appears only in association with the suppurative process in combination with the well-known causes of suppuration. Through its pigment it colors the pus blue, bluish-green, or green. In a series of cases the organism has occurred alone in connection with disease processes (otitis media, pericarditis, bursitis præpatellaris), so that it may very properly be looked upon as pathogenic for man, especially for children (Kossel). General septic infections are but rarely caused by this organism alone. Krannhals has collected some such cases (C. B. xv, 431); recently Escherich has described a small pyocyaneum epidemic among infants (C. B. xxv, 117). Its relation to diseases of children, where it is only found in the stools, remains doubtful (Baginsky).

Related Varieties.—According to our conviction, it is impossible to sharply separate this organism from the Bacterium fluorescens. Closely related also is a disagreeably smelling organism, cultivated by Galtier from a pig dead of a septic disease, and pathogenic for rabbits (C. B. IV, 109).

Schürmayer observed, as descendants of original cultures, forms which scarcely liquefy any more, representing short rods, forming tough coherent gelatin growths and a firm covering upon the liquefied gelatin. Many colonies in gelatin plates present marked, radiating striation (observed by us in Bact. fluorescens).

Bacterium fluorescens.[1] (Flügge.) Lehm. and Neum.

(Plate 25.)

Bacillus fluorescens liquefaciens. Flügge.

Literature.—Ruzicka (A. H. XXXIV, 148). Kurt Wolf: Die fluorescierenden Bacterien des Dresdner Ell- und Leitungswassers, Zeit. f. Gewässerkunde, 1898. Not accessible to us and only known to us through an abstract.

After the detailed description of the Bact. pyocyaneum it is unnecessary to also describe the Bact. fluorescens in detail, since we found it identical in all essential properties.

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[1] A transitional form to the following variety occurs in an organism which we obtained from A. Fischer as "termoähnlichen Bacillus." At first the gelatin remains solid, and liquefies very slowly after eight to fourteen days.

At first sight, absence of production of pyocyanin and of denitrifying action (very many cultures were investigated in vain in these respects by Weissenberg) appear sufficient to separate the organism from the Bact. pyocyaneum; but this is not the case, for the following reasons:

1. Ruzicka has also obtained Bact. fluorescens with formation of pyocyanin.
2. We and other writers have had cultures of Bact. pyocyaneum which no longer produce any trace of pyocyanin, and Ruzicka has observed, in aerated cultures of Bact. pyocyaneum, a marked reduction in the formation of pyocyanin (possibly transformation into pyoxanthose?).
3. Not only have Stutzer and Burri found a non-liquefying, fluorescent, denitrifying organism, but Künnemann claims to have cultivated from the soil, besides a denitrifying Bact. pyocyaneum, also a denitrifying Bact. fluorescens (C. B. L. IV, 906). Most recently Kurt Wolf has found the Bact. fluorescens to be frequently denitrifying (H. R., 1899, IX, 538).
4. The more restricted growth of the Bact. fluorescens in the stab canal as compared to Bact. pyocyaneum may be explained by acclimatization to higher temperatures; thereby also the pigment produced by the fluorescens takes on a bluer tone (Ruzicka).
5. Also the difference that the Bact. pyocyaneum, when introduced into the animal body, remains alive there very well, while the Bact. fluorescens after three days at the latest is dead, is not conclusive.

In short, the methodic investigations of Ruzicka agree absolutely with the impression which we obtained from our most careful comparison of the cultures, and which we advanced in the first edition.

We have studied most minutely four different cultures of the Bact. fluorescens isolated from water and soil.

Microscopically we found rods which were partly plump, and partly slender, with polar flagella.[1] Threads were rarely wanting. In Plate 25, VIII, a plump form is reproduced.

It stains poorly or not at all by Gram's method. Upon the nutrient media, we are unable, either microscopically or macroscopically, to see any difference between it and the Bact. pyocyaneum, except that milk is never coagulated, but rather clears up gradually, with a yellowish-green coloration. The yellowish-green ring about the growth on potato we have rarely seen. Usually a slight formation of indol is observed, but no H_2S. We have not conducted any experiments upon animals.

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[1] We are not acquainted with the non-motile **Bact. butyri fluorescens,** Lafar (A. H. XIII, 1), constantly present in Munich in butter. It does not change the color of agar.

The organism, with different variations of chromogenesis and fluorescence (yellowish-green, bluish-green, abundant, slight), is one of the most common inhabitants of water and soil, also it is very often found in milk, gastric contents, etc. The literature contains descriptions of a number of varieties claimed to be specific. We have not been able to study them, but are very skeptical regarding them because of the great variability of the Bact. fluorescens. E. Klein has cultivated from lupin tubercles a form which belongs here (Jour. of Path. and Bact., II, 1893, 205). (See p. 83.) Also **Bact. viridans** Symmers, from the vesicles of herpes (C. B. XII, 165), is entirely identical, in spite of its ability to grow also anaerobically.

Bacterium ranicida. (P. Ernst.) Lehm. and Neum.

Bacillus ranicida Ernst. (Ziegl. Beiträge, VIII, 203.) Bac. hydrophilus fuscus Sanarelli (C. B. IX, 193). (See also F. H. Russell, Jour. of Amer. Med. Assoc., June 18, 1898.—ED.)

Judging from the description and illustration of this organism, it appears to belong here. It is pathogenic for cold-blooded animals (frogs, fish), but, according to Sanarelli, also for warm-blooded animals. The rods are actively motile, and on many nutrient media grow into long threads. The cultures upon agar and gelatin exhibit a bluish fluorescence. Potato cultures are brown. They liquefy gelatin and ferment sugar, which was not done by any of the eleven fluorescent forms studied by us. The arrangement of the flagella may perhaps give further light upon their relationship.

Bacterium putidum. (Flügge.) Lehm. and Neum.

(Plate 26.)

Synonyms.—Bacillus fluorescens putidus Flügge, Bac. fluorescens non liquefaciens Autorum. Compare also remarks on page 285.

Microscopic Appearance.—Small, slender rods, often growing into exceedingly long threads. Thickness, 0.4–0.8 μ; length, 1.6–5 μ (26, VI, IX).

Motility.—Actively motile, dependent upon one, rarely two polar flagella.

Staining Properties.—Not by Gram's method.

Requirements as to Temperature, Oxygen, and Nutrient Media.—Strict aerobe, not particular as to media, grows fairly rapidly and best at 25°–30°.

Gelatin Plate.—(*a*) *Natural size. Deep:* Roundish to whetstone-shaped, yellowish. *Superficial:* At first like the deep; after forty-eight hours, 2 or 3 mm. wide, transparent, lobulated, ragged, shining, yellowish-green. The gelatin shows yellowish-green fluorescence (26, IV). It gradually enlarges until its size is 1 sq. cm.

(*b*) *Magnified fifty times. Deep:* Roundish, smooth-bordered, light yellow, homogeneously shaded, usually with a somewhat darker concentric ring (26, III). *Superficial:* Both in the early and later stages it is indistinguishable from the colonies of Bact. typhi and coli except from the fluorescence (26, II). There are here also manifold variations.

Gelatin Stab.—*Stab:* Not characteristic, thread-like. *Surface growth:* Lobulated, jagged, transparent, dull or with a fatty luster, whitish-gray to yellowish-green. The gelatin shows yellowish-green fluorescence (26, I).

Upon agar, potato, milk, and bouillon it is indistinguishable from Bact. fluorescens.

Remarks.—Aside from the liquefaction of gelatin, the Bact. putidum and Bact. fluorescens are scarcely different, and it appears entirely justifiable to place them together under a Bact. fluorescens, with forms α **liquefaciens** and β **non liquefaciens.** We have also reached the conclusion that the **Bacillus fluorescens albus** Zimmermann and **fluorescens longus** Zimmermann, which we received directly from Zimmermann and studied carefully, do not deserve to be designated as varieties. Both forms were identical with one isolated by us from soil; another, obtained from water, which we have cultivated for years in our institute, now forms very long threads almost exclusively, which we do not remember it to have done previously. A third form, isolated by us from soil, corresponds somewhat with the **Bacillus fluorescens aureus** Zimmermann, and is distinguished by a dirty yellow growth upon agar and gelatin, but this characteristic is not constant. Compare also Lesage (C. B. III, 8, and IV, 135) regarding the Bacterium of green diarrheas.

The same experience occurred to us with the Spirillum fluorescens of Král. It corresponded exactly upon all nutrient media with the Bact. putidum; microscopically it presented rods with a single flagellum, 0.4–0.6 μ thick and 0.8–3 μ long. We may here add that it may sometimes be very difficult to reach a certain decision as to whether we have to deal with a vibrio with a single flagellum, or a member of the fluorescent group with single flagella, since there occur almost straight vibrios as well as bent rods. At any rate, the fluorescent group forms the transition to the vibrios. The following appears to belong in this connection:

Bacterium denitrificans. Stutzer and Burri.

(L. and N.)

Bacillus denitrificans I. Stutzer and Burri. Liberates gaseous nitrogen from nitrite, and from nitrate only when reducing bacteria (Bact. coli and others) are present. For details regarding this interesting organism, see C. B. L. I, 257, and Weissenberg (A. H. XXX, 274).

Bacterium syncyaneum. (Ehrenb.) Lehm. and Neum.

(Plates 27 and 28.)

Literature.—Hüppe (Mitt. a. d. Gesundheitsamt II, 355), Heim (A. G. A. V, 518), Thum (A. K. I, 291).

Synonyms.—Bacillus cyanogenes Flügge, Pseudomonas syncyanea Migula. Bacillus of blue milk.

Microscopic Appearance.—Small rods, with blunt or pointed ends, 0.5 μ thick, 1.2–3 μ long. Threads could not be seen (27, VII).

Motility.—Active motion dependent upon from 1 to 5 flagella at one pole, rarely (before division) upon bipolar flagella (27, VIII).

Staining Properties.—With anilin dyes and by Gram's method. In staining plasmolysis sometimes occurs, so that the bacteria have stripes like a zebra.

Requirements as to Temperature, Nutrient Media, and Oxygen.—Obligate aerobe, grows best at room temperature, perceptibly less at 30°, and at 40° it soon dies. It grows with moderate rapidity.

Gelatin Plate.—(*a*) *Natural size. Deep:* Roundish to whetstone-shaped and yellowish. *Superficial* (after three days): Irregularly jagged lobulation, with a moist luster, a little elevated, sharply outlined from the surrounding medium, yellowish to grayish-white (28, VI). Later they become grayish to brownish-lavender. The gelatin is variously colored. (See also 28, VII.)

(*b*) *Magnified fifty times. Deep:* Round or roundish, yellowish, delicately granular (28, VIII i). *Superficial:* In the youngest stages are not distinguishable from those of Bact. typhi and coli. Also later they are still very similar to them, only the colonies appear much more delicately granular. Often the original colony appears at the middle as a yellowish-brown nucleus. Every possible variation of form, structure, and color is observed. The color usually is yellowish and the form irregularly lobulated (28, VIII e).

Gelatin Stab.—*Stab:* Not characteristic, thread-like. *Surface growth:* From whitish and bluish-gray to greenish-yellow, with a moist luster, slimy. The color of the gelatin varies exceedingly. A culture obtained from Berlin in the summer of 1895 usually furnished light to dark blue growths, while a culture of our own, which had been cultivated in the institute for about six years, exhibited, upon the same nutrient medium, brownish-green, dark brown, and light yellowish-green growths with more or less fluorescence. A Bact. syncyaneum β cyaneofluorescens Zangemeister (C. B. XVIII, 321) behaved very similarly. A year later also the Berlin culture produced no blue color upon either acid or alkaline nutrient media, but only dirty colors, from light or dark brown to light yellowish-green and deep brownish-green (27, I, II, III). (Compare also 27, IV.) A culture freshly isolated from milk in Würzburg gave beautiful blue pigment with little fluorescence.

Agar Plate.—(*a*) *Natural size:* Like those on the gelatin plate.

(*b*) *Magnified fifty times. Deep:* Roundish to whetstone-shaped, yellow, gray, or brownish, with even border, homogeneous, shaded. *Superficial:* Round to roundish, smooth edge, light yellowish to grayish-brown, homogeneously shaded or finely granular. It is similar to the colony of Bact. fluorescens (28, V).

Agar Stab.—Just like that in gelatin. Usually the surface growth is a little more luxuriant (27, IV). Compare 27, I–III.

Agar Streak.—Moist, usually grayish-white growth, with a smooth, wavy border. Water of condensation cloudy, with a grayish-white sediment. The agar presents most variable colors. Sometimes cultures can not be distinguished from those of the Bact. putidum.

Bouillon Culture.—Moderately cloudy, at first grayish-green, later with many cultures, becoming blue to bluish-green. Precipitate moderate, whitish-gray, feebly coherent. Pellicle formation is observed in some cases, and not in others (27, V).

Milk Culture.—Bluish-green color; otherwise unaltered. Reaction alkaline (27, VI). With the addition of hydrochloric acid the color becomes blue, if a culture is employed which forms syncyanin. Upon unsterilized milk the color is deep to sky blue on account of the acid produced by the Bact. acid. lactici.

We obtained beautiful blue milk by adding 1% grape-sugar to sterilized milk or, better, whey; grape-sugar, particularly, is converted into acid by Bact. syncyaneum.

Potato Culture.—According to the variety of potato, growths resulting from inoculation with the same culture may vary widely. The growth is greenish or brownish-blue, blackish-blue, dark brown, yellowish-brown, or gray, always glistening, sometimes a little elevated. The potato is discolored greenish, brown, gray, blue, etc. (28, I–III). In many cases it can not be distinguished from Bact. fluorescens, especially if the formation of blue pigment is slight or absent.

Other Nutrient Media.—Grows and produces pigment upon non-albuminous nutrient media. As pointed out by Hüppe, tartrate of ammonium serves as the source of carbon and nitrogen.

Resistant Properties.—Against drying, five to seven months (Heim). Spores are certainly not present (Heim).

Chemical Activities.—*Chromogenesis:* Most cultures form two pigments, the fluorescent yellowish-green bacteriofluorescein, and also the blue syncyanin. We have possessed cultures which no longer produced any trace of blue pigment, but only bacteriofluorescein, and Hüppe and Thumm (A. K. I, 291) have observed those which produced a pure blue pigment. Finally, all chromogenesis may be lost (Heim, *l. c.;* Behr, C. B. VIII, 485). Regarding syncyanin, we still know little; we have been unable to find a good solvent for it; spectroscopically it furnishes a powerful absorption band. Weak acids do not alter the color, strong hydrochloric and sulphuric acids change it to violet, acetic acid produces a dirty color. Sodium hydroxid produces a rosy to yellowish red; the change in color occurs as the acid reaction disappears; upon standing, the color passes into brownish-red, thus explaining the color of old cultures.

Acid is not formed from milk-sugar, but is from grape-sugar without liberation of gas. In peptone bouillon no H_2S is formed, but traces of indol are produced. The bouillon smells disagreeably aromatic, and abundant ammonia is formed.

Distribution.—(*a*) *Outside the body:* Often found in blue milk, sometimes occurring epidemically. Such milk is not harmful. (*b*) *In the body* the organism has not been found.

Bacterium brunificans. Lehm. and Neum.

Actively motile, does not liquefy gelatin, isolated from foul pus by Scheibenzuber (C. B. VI, 441). In stab cultures upon various nutrient media it discolors the medium dark brown in the form of a sack—*i. e.*, above for a short and below for a greater distance from the stab canal. Upon the potato there develops a brown growth, about which is a ring of dark brown discoloration.

Bacterium ferrugineum. (Rullmann.) L. and N.

According to the description, it is closely related to the preceding. It is actively motile. Cultures yellowish or reddish, but usually dark brown, with marked rusty brown discoloration of the nutrient medium. Pigment is soluble in water, alcohol, and acetone. Upon meat infusion-glycerin-agar there is, at 37°, a greenish fluorescence. Gelatin is feebly liquefied. Found by Rullmann in canal-water (C. B. XXIV, 465).

Bacterium Zopfii. Kurth. (Botan. Zeit., 1883.)

(Plates 29 and 30.)

Microscopic Appearance. — There occur all forms from long threads to short rods. Often the threads break up into nearly spherical members (30, II).

Motility. — Very active, dependent upon numerous peritrichous flagella (30, IX).

Staining Properties.—Stains well by Gram's method.

Requirements as to Nutrient Media, Temperature, and Oxygen.—Facultative anaerobe, satisfied with the greatest variety of nutrient materials; grows at room and incubator temperatures.

Gelatin Plate.—(*a*) *Natural size:* Delicate, whitish-gray colonies, resembling spider's web or mold mycelium (29, VI). Later there appear little branches upon the threads, which are more distinct the more superficially they are located. The colonies (29, V) then resemble those of the Bacillus mycoides (37, VI, IX).

(*b*) *Magnified 50 to 100 times:* Very characteristic. The original colony serves as a central part, from which radiating threads pass outward on all sides, which are more or less branched and matted together. Between these lie zoogleæ of the most variable forms: resembling loops of hair, corkscrews, whipcords, and sausage with marked reflection (29, VIII). When magnified 90 times, the individual threads appear as wavy strings with wide lumina, arranged in a most irregular manner (29, VII). The ribbon-like zoogleic forms, when magnified 90 times, appear composed of highly refractive, often granular threads (30, I). The elongated, sausage-like forms appear decidedly irregular. They consist of oval, overlapping, yellowish-gray, homogeneously shaded clumps. At the end of such a chain there are usually bough-like branchings. Between these lie younger zoogleæ, bounded by two notched lines (30, VII).

Gelatin Stab.—The stab is provided with very delicate, fine, parallel outgrowths, which are longest near the surface and become shorter as they become deeper in the tube. Surface growth is delicate, transparent, gray, shining, sometimes presenting very beautifully a combination of small boughs (29, I).

Agar Plate.—(*a*) *Natural size:* After twenty-four hours the colonies are 2 to 4 mm. wide, grayish-white, with delicately fringed border, and are surrounded by a thin transparent zone (30, IV). In a short time the whole plate is covered with a gray veil.

(*b*) *Magnified fifty times:* After twelve hours the colonies are like exceedingly delicate balls of hairs, and visible only with a very narrow opening in the diaphragm (30, V). Later the colonies take on a more intense yellowish color, the branching increases, and the extension takes place rapidly but irregularly. The colony can not be distinguished from a deep subtilis colony (30, III). After a few days the colony has become yellowish-brown, and is exceedingly matted and tangled. When magnified 90 times, it is seen that the delicate veils about the superficial colonies consist of thin layers of bacteria (30, VI).

Agar Stab.—Similar to that in gelatin (29, III).

Agar Streak.—Exceedingly delicate, grayish-white, transparent, shining. In the middle there sometimes is a paler streak. In a short time the entire surface is overgrown; hairs are not usually distinguishable; the water of condensation remains clear, with a whitish precipitate.

Bouillon Culture.—Clear or slightly turbid, with a little sediment.

Milk.—Not coagulated, amphoteric reaction.

Potato Culture.—Slight, yellowish-gray growth.

Chemical Activities.—Produces typical putrefaction with pronounced foul odor upon nutrient media rich in albumin. It is remarkable that Kuhn (A. H. XIII, 40) could never demonstrate indol production in our institute, while we now find some indol.

Distribution.—(*a*) *Outside the body:* Isolated by Kurt from hen dejecta; by Kuhn, repeatedly from putrefying mixtures. (*b*) *Inside the body* it has never been found.

Related Varieties.—The Bact. vulgare, forma Zenkeri, which has so far been but little studied, is most closely related. The principal difference lies in the beautiful little hairs, bristles, and threads sent out from the stab culture. According to Hauser's description, it appears that also the elongated, sausage-shaped zoogleæ are lacking in his Proteus Zenkeri. In Kuhn a confusion of our organism with the Prot. Zenkeri is met with; in his work it is everywhere called Bact. Zopfii instead of Proteus Zenkeri (A. H. XIII, 40).

Bacterium vulgare. (Hauser.) Lehm. and Neum.

(Plates 31 and 32.)

Synonyms.—*Proteus vulgaris* Hauser, *Bacillus vulgaris* Macé, Migula. *Proteus Hauseri* Autor., *Bacillus albus cadaveris* Strecker and Strassmann (C. B. IV, 67), *Urobacillus liquefaciens septicus* Krogius, *Bac. fœtidus ozænæ* Hajek, *Bacillus Proteus vulgaris* Kruse.

Ordinary Name.—Proteus.

Literature.—Hauser, "Ueber Fäulnisbakterien," Leipzig, 1885. Meyerhof (C. B. XXIV, 18), extensive review of literature (152 numbers).

Microscopic Appearance.—Slender, thin rods, averaging 1.6–4 μ in length, and 0.4–0.5 μ in thickness. It is often found as long threads, but it also occurs in isodiametric forms and as spiral winding threads. The multiplicity of the microscopic growth-forms has led to naming the organism as Proteus. Upon acid nutrient media, very short rods are especially produced (31, VIII and IX).

Motility.—Very active, due to very abundant, long, peritrichous flagella. At present our cultures exhibit active motion only when examined while very young, in spite of a good development of flagella (31, IX).

Staining Properties.—Stains well by Gram's method. When treated by Gram's method, it was found by Meyerhof to be easily decolorized (C. B. XXIV, 27), and by Silberschmidt to be unstained. We have demonstrated anew with many cultures that it stains well.

Requirements as Regards Oxygen and Nutrient Media.—It grows equally well aerobically and anaerobically, also in CO_2. The most variable nutrient media (also non-albuminous) are suited to it. It grows very rapidly. Hauser found that, in the absence of oxygen and in carbonic acid, growth was poor, also upon non-albuminous nutrient media. It grows at quite low temperatures (ice-box) and in the incubator. According to Levy and Meyerhof, gas production is most abundant when oxygen is freely admitted, as when the bacteria are grown in shallow dishes.

Gelatin Plate.[1]—(*a*) *Natural size:* Gray, delicate, transparent growths which present a shallow depression even after twelve to twenty hours. After three days the cups of liquefaction are already 0.5 to 1 cm. wide, with turbid contents. The deep colonies are punctiform and not characteristic. Before liquefaction there is usually seen about the superficial colonies an irregularly jagged zone, consisting of highly refractive zoogleæ (31, v).

(*b*) *Magnified sixty times:* Upon very young plates two kinds of colonies are seen—the one, roundish, grayish-yellow, sharply outlined, with even borders, homogeneous or finely granular, which lies deep in the gelatin; the other, transparent, colorless, delicate, with wavy lobulations, difficult to distinguish from those of Bact. typhi, which lie upon the surface. The latter spread out more and more, and then there appears in the center of the colony a lively, interesting motion of the bacterial masses. After a longer time the motion ceases, while liquefaction extends at the periphery. The colony then is of an irregular form, and when the entire colony is almost completely liquefied, the thin shining peripheral portion remains. The deep colonies often present hairs, which later are mostly arranged about the periphery.[2]

Gelatin Stab.—*Stab:* At first is thread-like and not characteristic; later, there is tube-shaped liquefaction. The *surface growth* at once forms a saucer-shaped liquefaction, and later the liquefaction becomes cylindric. The liquefied material is turbid or cloudy (31, I). (The picture is a little too violet.)

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[1] Sugar gelatin is not liquefied, the growth upon this medium being entirely similar to that of the Bact. Zopfii, but in the stab the outgrowths are absent (Kuhn).

[2] The representation given is taken from a culture which has been long cultivated and often studied. Not rarely, especially in freshly isolated cultures, one observes on gelatin plate cultures sausage-shaped, spiral zoogleæ exactly identical with those which we have described and illustrated so minutely in the Bacterium Zopfii, and which Hauser has so beautifully photographed. Schedtler (C. B. II, 437) appears to have submitted cultures similar to those represented by us. We have sometimes noticed the swarming islands at the periphery of the colonies, which, according to Hauser, are especially observed upon 5% tin.

Agar Plate.—(*a*) *Natural size:* Entirely non-characteristic. The surface colonies are delicate grayish-white; the deeper, yellowish-white (31, III).

(*b*) *Magnified sixty times. Deep colonies:* Roundish, very crumbly, later often moruloid (33, IV below). *Superficial:* Delicately transparent, exceedingly finely granular, at the center yellowish and becoming colorless toward the periphery. The periphery assumes all possible irregular forms, from the wandering outward of the bacteria (32, VII). At first it is always roundish (31, IV). Typical, elongated, sausage forms, etc., such as occur on gelatin, have never been observed by us.

Agar Stab.—*Stab:* Not characteristic, thread-like. *Surface growth:* Gray, slimy, moist, transparent.

Agar Streak.—Veil-like, thin, transparent, moistly glistening growth, which already after twelve hours has overgrown the entire surface. Water of condensation very cloudy, whitish-yellow.

Bouillon Culture.—Very cloudy, abundant precipitate.

Milk Culture.—Firmly coagulated after two to three days, and later again liquefied. Still later, milk becomes yellowish and feebly acid.

Potato Culture.—Very scanty growth; whitish-yellow, usually limited to the inoculation streak, somewhat crumbly, dull or with a fatty luster, somewhat-elevated.

Chemical Activities.—

(*a*) *Odoriferous substances:* Albuminous bodies undergo putrefactive decomposition with very abundant foul odor, and a strong alkaline reaction.

(*b*) *Formation of gas and acid from carbohydrates:* It forms abundant gas from grape-sugar; according to Th. Smith, still more from cane-sugar, and none from milk-sugar. According to Smith, the gas consists of one-third CO_2 and of two-thirds H_2. Upon sugar media no foul odor is present (Kuhn).

(*c*) H_2S and indol are produced abundantly.

(*d*) Urea is vigorously transformed into ammonium carbonate. (Compare p. 70.)

(*e*) *Toxins:* Hauser observed the production of exceedingly powerful toxic metabolic products, which could be obtained free of bacteria by passage through clay filters. Tito Carbone has isolated cholin, ethylendiamin, gadinin, and trimethylamin from meat cultures (C. B. VIII, No. 768).

The sepsin of Schmiedeberg, from putrid yeast (Mediz. Centralblatt, 1868, No. 32), acts just like the metabolic products of the Bact. vulgare (Levy), and appears to be a product of that organism.

Resistance.—Considerable against chemical and thermal injuries, but is killed by 60° in one-quarter to one-half minute (Meyerhof).

Distribution.—

(*a*) *Outside the body:* Very common in putrid meat and other putrid objects. Cause of foul-smelling decomposition; occurs in water from contamination with putrid materials. It never occurs in gelatin plates from air, but is easily obtained if sterile or sterilized meat is allowed to stand uncovered; thus it is present in air.

(*b*) *In healthy body:* Throughout the entire alimentary tract.

(*c*) *In diseased human organism:* Often alone it produces severe catarrh of the bladder with ammoniacal urine; often, also, in association with Bact. coli (Schnitzler, C. B. XIV, 218). It is also the cause of other diseases of the urinary organs. The urobacillus liquefaciens septicus of writers is at least in part identical with the Bact. vulgare.

While Bact. vulgare occurs rather frequently together with other causes of disease (in foul phlegmons, abscesses, pulmonary gangrene, decubitus, foul carcinomas, etc.), it has relatively seldom been demonstrated to certainly be the cause of diseases in man, as in a few cases of abscess, inflammations of serous membranes, etc.

Booker found varieties of the proteus in 18 cases of cholera infantum (C. B. x, 284).

Levy has demonstrated the Bact. vulgare to be the cause of meat poisoning: Eighteen persons were taken sick with severe vomiting and diarrhea (vomiting of blood), of whom one died. Compare also the epidemic described by Wesenberg (Z. H. xxviii, 484).

In an instance where several soldiers became very sick with Weil's disease (infectious, febrile icterus) after bathing in impure water, Jäger (Z. H. xii, 525) found the Bact. vulgare in a feebly fluorescent form; in two cases postmortem, in great numbers in some of the organs; in four out of six lighter cases examined, in the urine. It was also possible to demonstrate that the same organism was present in the bath water, which, besides, caused a sickness in fowls. Jäger pointed out the great variability of his organism, and believes that sometimes the Bact. vulgare may be actively pathogenic. Also a series of other cases of infectious icterus are to be referred to proteus infection—whether all, is questionable.

Experimental Observations Regarding Pathogenic Properties.—Hauser did not obtain true infection; his animal experiments are all intoxications with the metabolic products (dyspnea). Meyerhof has produced, with large quantities of slightly virulent proteus cultures, fatal disease in mice, rabbits, and dogs, accompanied by an increase of the bacteria introduced, it thus being a true infection. The filtrate of the cultures was very weak, devitalized (chloroform) cultures having but little effect.

Virulent forms of proteus, when injected subcutaneously in an animal (rabbit), produce putrid abscess. This occurs much more easily if other organisms (for example, streptococci) are simultaneously introduced into the body. Slightly virulent, pathogenic varieties (staphylococci, streptococci) increase in virulence if they are injected simultaneously with dead or living proteus cultures.

O. Wyss has convincingly demonstrated Bact. vulgare to be the cause of a disease in fish (Z. H. xxvii, 143).

Immunity and Serum Reaction.—According to Carbone, it is possible to immunize animals against the living bacteria by means of their metabolic products. According to Pfaundler, the serum of animals which pass through an afebrile proteus infection agglutinates the proteus individuals of the same stock. If, however, the sickness is accompanied by fever, then no agglutination occurs, but in such a serum the organism grows out into long threads. Similar results—the serum being active only or especially against the particular culture employed to produce the sickness or immunity—were obtained also by others. Compare S. Wolf (C. B. xxv, 317).

Related Varieties.—Hauser (*l. c.*) has designated with the name Proteus mirabilis a form of the Bact. vulgare characterized by somewhat more feeble liquefaction, and producing especially striking involution forms. Another he has designated as Proteus Zenkeri, which does not liquefy gelatin and does not any longer cause vigorous putrefaction. These forms were later recognized by Hauser as transformable into each other (C. B. XII, 629). Here also belongs Gerdes' eclampsia bacillus. On the contrary, a Proteus hominis Bordoni-Uffreduzzi (Z. H. III, 333) certainly belongs among those related to the Bact. pneumoniæ; it does not exhibit motility, formation of zoogleæ, nor the production of putrefaction. (Compare p. 228.) The case may be considered as similar to Banti's four "varieties of proteus" (C. B. v, 207).

Bacterium murisepticum. (Flügge.) Migula.

(Plate 33.)

Synonyms.—Bacillus murisepticus Flügge. Bacillus of mouse septicemia Koch.

Literature.—Koch, R., Wundinfektionskrankheiten, p. 40; Preisz (C. B. XI, 110); Löffler (C. B. XI, 129).

Microscopic Appearance.—In the culture, beautiful, slender rods, 2–4 μ long, 0.4–6 μ thick, straight or curved, often arranged in threads (33, VIII). In preparations of smeared blood the organisms are only about 1 μ long and 0.2–0.3 μ thick (33, IX).

Motility is absent.

Staining Properties.—Stain well by Gram's method.

Relation to Oxygen.—Facultative anaerobe. Liborius found it an obligate aerobe. Many cultures grow decidedly better when air is excluded.

Intensity of Growth.—Grows rather slowly.

Gelatin Plate.—(*a*) *Natural size:* After three or four days there is a very shallow depression, in which the colony rests as an exceedingly delicate veil, only distinguishable from the surrounding medium with difficulty (33, v). The plate represents the colonies as too distinct.

(*b*) *Magnified fifty times:* The colony is visible only with a very narrow opening in the diaphragm. It is not without difficulty that one observes the exceedingly slight, delicate, gray growth of homogeneous or finely granular character and differentiated from the surroundings with little sharpness (33, vi). When magnified 90 times, there is seen what suggests a tangle of threads. Other writers—for example, B. Preisz—describe the colonies as somewhat denser; from a homogeneous or matted nucleus there radiate outward ramifying and interlacing threads, which sometimes wind like a corkscrew.

Gelatin Stab.—After a few days the stab canal represents the structure of an exceedingly delicate fir-tree, with branches of equal length throughout the entire length (33, iii), which after a longer time become in part more and more confluent and remain as delicate, transparent clouds in the gelatin. Upon the surface there gradually forms a slight, pointed depression. The atypical anthrax culture (34, v) presents a similar, but very much coarser picture.

Agar Plate.—(*a*) *Natural size:* Small, very insignificant, whitish-gray points, which are barely visible only when examined upon a dark background.

(*b*) *Magnified fifty times. Superficial:* At first gray, delicate, veil-like; later, more brownish or yellowish. The homogeneous structure becomes finely or moderately coarsely granular and sometimes looks not unlike the granulation of a finely granular sarcina. *Deep:* Roundish to whetstone-shaped, yellowish, homogeneous (33, vii), with a smooth or granular border.

Agar Stab.—Similar to that in gelatin, a little less luxuriant. The branches may be entirely absent. Surface growth exceedingly delicate, transparent, spreading but little, colorless. Sometimes the growth is indicated only by a little luster (33, iv).

Agar Streak.—Delicate, exceedingly thin growth (33, ii).

Bouillon Culture.—No pellicle, little turbidity, very little sediment, which is very slightly coherent.

Milk Culture.—No coagulation, amphoteric or feebly alkaline reaction.

Potato Culture.—No perceptible growth.

Chemical Activities.—No formation of pigment or odoriferous substances. Our culture produces no H_2S or indol. Petri and Maassen observed vigorous production of H_2S. A little acid is formed from grape-sugar and gelatin is very slowly liquefied.

Distribution.—

(*a*) *Outside the body:* Isolated repeatedly by Koch and others from canal-water and putrid mixtures (putrid meat, yeast).

(*b*) *In the body:* Not found in man, for whom the organism is not pathogenic. It causes mouse septicemia, an artificial infectious disease, discovered by Koch, which also occurred spontaneously in one instance in Greifswald.

Special Culture Methods.—Inoculation of a white mouse with the suspected material. Stained smear preparations, plates, and stab cultures are made from the blood and spleen, where the bacteria are present in abundance.

Pathogenic Effects upon Animals. — Pathogenic (death in two or three days) for house mice (not for field mice). *Symptoms:* Eyelids stuck together, head drawn in, a tendency to sleep. Also pigeons die in two and one-half to three and one-half days (Th. Smith). Rabbits and guinea-pigs withstand large quantities of bouillon culture. In swine it produces only transitory indisposition.

Bacterium erysipelatos suum. (Löffler.) Migula.

(Swine Erysipelas *pro parte*, Bacillus of Erysipelas of Swine.)

Bacillus rhusiopathicæ suis Kitt.

Literature.—Löffler (A. G. A. I, 46). Preisz (C. B. XI, 110).

This organism is very closely related to the one causing mouse septicemia, being identical microscopically, and the stab culture is extremely similar, only the branches are a little more sturdy and bristly (33, I). When the inoculation is made in gelatin from the blood, according to Lorenz, the branches are sometimes almost entirely absent from the primary cultures, and, instead, only nodules and globules are seen in the stab. The principal difference lies in the gelatin plate colonies, which were described by Löffler as minute, distinctly visible growths, with a few irregular radiations (like bone corpuscles), and which were found by us to be constantly somewhat between 31, VI, e, and 31, VII, on an average. Compare Lösener, A. G. A. XII, 448. There is intense production of H_2S, and little of indol. Some acid is formed from grape-sugar.

Regarding a rather considerable resistance to pickling and smoking, consult Petri (A. G. A. VI, 266).

It causes an important disease of swine, young animals of the choicer varieties being especially affected. Older animals, and also younger ones of ordinary breeds, are more or less immune. Upon section the animals show, besides a patchy or diffuse redness of the skin, which is often very marked, also subcutaneous edema, redness of the pharynx and of the gastric and intestinal mucosa, swelling of the mesenteric glands and spleen, parenchymatous nephritis, hemorrhages in the kidneys, and red spots in the lungs. Consult Graffunder, Berl. tierärztl. Wochenschrift, 1896, No. 2.

The organism is not pathogenic for man, also the flesh of swine affected with erysipelas is harmless.

Mice sicken and die after feeding, and more rapidly after inoculation; also rabbits usually succumb to inoculation.

The differential diagnosis from other diseases of swine is easy, from the characteristic form of the individuals and from the cultures of the organism. It must not be forgotten that redness of the skin in patches occurs in many diseases of swine, as in Löffler-Schutz's swine plague (see p. 254).

Protective Inoculation.—Animals may be actively immunized with attenuated bacteria (Pasteur), with devitalized bacteria, with body juices (Emmerich) and blood-serum (Lorenz, C. B. XIX, 168).

According to Voges and Schütz (Z. H. XXVIII, 38), no method has stood practical tests. The serum of actively immunized animals possesses active bactericidal substances. In the place last referred to is also found an extensive review of the literature.

Bacterium of Brick-pock (Backsteinblättern). Lorenz (C. B. xi, 672).

A disease of swine,—evidently to be considered as a form of swine erysipelas,—which almost always runs a favorable course, has been described by Lorenz as brick-pock, and he has ascribed it to an organism which, upon subcutaneous inoculation, stands midway, as to virulence for swine, between mouse septicemia and swine erysipelas. After inoculation with it, swine become immune to swine erysipelas. Rabbits, on the contrary, in a very interesting manner are much more susceptible to brick-pock than to swine erysipelas. They always succumb to brick-pock infection, but may be immunized with swine erysipelas against brick-pock. Lorenz likewise holds that swine erysipelas, mouse septicemia, and brick-pock are produced by forms of one organism, even if the transformation of one form into the other has not been entirely successful.

2. Bacillus F. Cohn, emend. Hüppe.

Straight rods, often growing into threads, often of considerable thickness, rarely less than 0.6, usually more than 0.8 μ. They form endospores.

Key to the Recognition of the More Important Varieties of the Genus[1] Bacillus.[2]

I. Aerobic Varieties, thriving only scantily anaerobically. The pathogenic ones never form spores in the animal body, but only in cultures with oxygen admitted (compare also p. 310). Almost all in cultures grow into long threads with central spores.

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[1] Regarding our insufficient knowledge of this genus, consult the statements upon page 306 and in the discussion of the anaerobes. Numerous new aerobic varieties are described by Burchard (A. K. II, 1).

[2] The genus **Tyrothrix** Duclaux is included in the bacilli. It designates primarily varieties originating from milk and cheese, which form spores and grow into long threads. Two species are described below: Bac. tenuis and Bac. geniculatus. The most remarkable statements of W. Winkler regarding extraordinary biologic and morphologic variability, especially in the Bact. tenuis (C. B. L. I, 657), could not be con[illegible] by ourselves (see first edition) or by

(*A*) *Stab culture in gelatin with projecting branches:*

1. Branches distinct, usually only in the upper part of the stab. Agar plate colonies, when magnified 60 times, have beautiful regular curls. Agar streak culture without branches, wide, white with "silvery vesicles." Never motile. Pathogenic for animals. **Bac. anthracis** Cohn and Koch (p. 307).

2. Branches delicate, extending along the entire length of the stab. When magnified 60 times, the colonies in the agar plate exhibit irregular outgrowths in the form of roots or the mycelium of molds. Agar streak culture has long, delicate, parallel transverse branches. Sluggishly motile. Not pathogenic for animals. **Bac. mycoides** Flügge (p. 316).

(*B*) *Stab culture in gelatin without projecting branches; motility dependent upon peritrichous flagella:*

1. Potato growth at first moist and flat, later (about eight days) with a distinctly mealy sprinkling. **Bac. subtilis** Cohn (p. 317).

2. Potato growth moderately elevated, not characteristic, resembling Bact. coli. **Bac. oxalaticus** Zopf, **butyricus** Hüppe, **megatherium** De Bary (pp. 321, 322, and 323).

3. Potato culture luxuriant, moist, intensely yellow. Agar moist, mustard yellow. Later resembles vulgatus. **Bac. luteus** L. and N.[1]

4. Potato is not characteristic during the first days; later, there forms a distinct, wrinkled elevation.

(*a*) The folds are padded, like coils of intestine. **Bac. vulgatus** (Flügge) Migula (p. 323).

(*b*) The folds are low, reticulated. Growth yellowish. **Bac. mesentericus** (Flügge) Lehm. and Neum. (p. 326).

(*c*) Growth moist, wrinkled, besides the potato is deep black. **Bac. aterrimus** Lehm. and Neum. (p. 328).

(*d*) Growth rose-colored, a little wrinkled, gelatin smoky brown. Compare also Bac. mesentericus ruber. **Bac. gangrænæ pulpæ** Arkövy (L. and N.) (p. 328).

5. The potato growth is delicate, syrupy, clear. **Bac. liodermos** (Flügge) Lehm. and Neum. (p. 328).

II. ANAEROBIC VARIETIES (of which, certainly, partially aerobic forms exist). Only exceptionally form long threads. Staining by Gram's method rarely well developed (the Bac. tetani stains well). Spontaneous motion dependent upon peritrichous flagella is rarely lacking. The spore is usually located at the end (paraplectrum form) or at the middle, usually with some bulging (clostridium form). In most varieties both forms of sporulation occur. The recognition of the individual varieties, which is often very difficult, and even impossible, may be rendered somewhat easier by means of the following scheme:

(*A*) *Pathogenic Varieties.*—

Wittlin (C. B L. II, 475); and since then, so far as we know, it has not been upheld.

[1] For more details regarding this organism, compare Bacillus luteus sporogenes Wood Smith and Baker (B. C. L. IV, p. 788).

1. In a deep cutaneous pocket in animals no local symptoms are produced, but only or preponderantly nervous symptoms.[1]

(*a*) Produces tetanus. **Bac. tetani** Nicolaier (p. 332).

(*β*) Causes symptoms of botulism: disturbances of the innervation of the pupils and accommodation, aphonia, paresis in the region of the tongue and pharynx, disturbances of the salivary and mucous secretions, etc. **Bac. botulinus** v. Ermengem (p. 337).

2. When introduced into a deep cutaneous pocket in animals, it causes local bloody, often emphysematous edema. The organism spreads in the body, especially in the edema. Guinea-pigs are especially susceptible.[2]

(*a*) Motile.

(*α*) In the edema growing into long, jointed threads, usually not present in the bile. Very pathogenic for rabbits. Brain nutrient media darkened. Not stained by Gram's method. **Bac. œdematis maligni** (Koch) Flügge (p. 341).

(*β*) No long threads in edema; usually only pairs. Usually slightly pathogenic for rabbits and mice. Always found in the bile. Brain nutrient media not darkened. Usually stained by Gram's method. **Bac. Chauvœi** of French authors (p. 339).

(*b*) Not motile. Disease picture similar to symptomatic anthrax, but there is a tendency to grow into long threads. **Bac. phlegmonis emphysematosæ** E. Fränkel (p. 344).

3. Only known as injurious to bees. **Bac. alvei** Chesire and Cheyne (p. 345).

(*B*) *Zymogenic Varieties.*—

A large group which has not yet been sufficiently cleared up. Compare page 345, etc.; also especially page 348. Here belong many forms producing butyric acid.

Introductory Remarks to the Special Description of the Aerobic Varieties Here Given.

(Common Characteristics.)

All of the varieties described in what follows,—Bac. anthracis, mycoides, subtilis, megatherium, butyricus, vulgatus, mesentericus, aterrimus, liodermos,—which are very closely related to each other, have the following biologic properties in common, which may be given here for all of them:

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[1] Non-pathogenic cultures cannot always be diagnosed with certainty from the morphologic and biologic properties.

[2] Compare also the Bac. sporogenes (Klein) L. and N. (p. 346), which occupies a place midway between malignant edema and symptomatic anthrax; also the pseudoedema bacilli (p. 343).

1. Gelatin is liquefied.
2. Milk is alkaline or very feebly acid in reaction, is coagulated, and later the coagulum is dissolved.
3. All form little acid from grape-sugar (see Table I, at end of the book, for quantitative statements) and no gas. From milk-sugar there is formed little or no acid.
4. No indol is formed. The production of H_2S is variable, never abundant.
5. All are stained by Gram's method.

The equipment with flagella appears also in this group to be valuable for the diagnosis of species only with great precautions. When present, they are peritrichous.

Bacillus anthracis. F. Cohn and Koch.

(Plates 34, 35, and 36.)

Ordinary Names.—Anthrax bacillus, Bactéridie du charbon.

Microscopic Appearance.—In the animal body it occurs as large vigorous rods, 3–10 μ long, 1–1.2 μ thick, which are often arranged in longer or shorter chains (36, I). The ends in fresh specimens are a little projecting (rounded); after drying and staining, they appear square-cut or slightly concave. To demonstrate the capsules,—which are always well developed in the animal body, fluid blood-serum, and brain-agar mixture,—the directions given in the technical appendix are to be followed. According to Kern, capsules may be demonstrated in old cultures upon most variable nutrient media.[1]

In artificial nutrient media the bacilli grow into long threads, placed parallel or somewhat twisted and entangled (36, II), which either produce spores (see below) or perish in the formation of bizarre involution forms (36, V). The threads, even when unstained, give indications of their being composed of separate bacilli (36, VI). This is especially distinct after staining.

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[1] Noetzel has also demonstrated capsules in undoubted "cadaver bacilli," and this points out how unsafe it is to allow the diagnosis of anthrax to rest upon the demonstration of capsules, which is often very much overestimated by veterinarians. (C. B. XIX, 498.)

Motility.—Always is absent. To this no exception is known.

Staining Properties.—Stains with all anilin dyes and by Gram's method.

Relation to Oxygen.—Grows best when oxygen is admitted. When oxygen is excluded, it grows poorly and without liquefaction. There is no growth in CO_2.

Intensity of Growth.—Grows rapidly, especially at 37°. Lower limit of growth at 14° (Kitasato).

Gelatin Plate.—(*a*) *Natural size. Superficial colony:* Whitish, round; after three or four days, deeply sunken. Also, upon longer standing the liquefaction only extends slowly. In the middle of the abrupt crater there lies a white, crumbly, poorly defined mass, the remainder of the contents of the liquefied area being rather clear, but the outermost peripheral zone is somewhat turbid again (35, V).

(*b*) *Magnified seventy times:* The colonies when three days old appear distinctly darker than on agar. Near the center grayish-yellow, toward the edge more distinctly transparent. At the periphery the formation of locks is clearly seen, but toward the center they become very dense and indistinct (36, VI). The liquefaction is recognized as a grayish reflex. Later an irregularly outlined ball, devoid of distinct locks, floats in the liquefied medium.

Gelatin Stab.—Along the stab there forms a thick white thread, from which, as a rule, only in the upper part (34, II), more rarely throughout the entire length, long (34, I) or short (34, III), bristly, distinct outgrowths extend outward. Sometimes the growth of hairs fails entirely (34, IV). Also the direction of the lateral outgrowths varies; many times they are tangled together (34, V). After twelve to twenty hours there begins a slowly progressing liquefaction, with limited depression of the surface of the gelatin. The liquefaction at first is cup-shaped, later cylindric. The content of the funnel is sometimes diffusely cloudy with white crumby flocculi; at other times the flocculi settle down, leaving a clear liquid gelatin above. No pellicle is ever formed.

Agar Plate.—(*a*) *Natural size. Superficial colonies:* Small, white, with a play of yellow, moistly shining, a little elevated, roundish. *Deep:* Punctiform and permanently small (35, II). The structure is the same as given for the agar streak.

(*b*) *Magnified fifty times:* Deep and superficial colonies present great differences. The former are usually whetstone-shaped, roundish, greenish-gray, becoming yellowish toward the center. The peripheral zone consists of coarse, dark-colored, crumbly masses, which are continued as shorter or longer outgrowths, composed of little hairs, crumbs, and granules. If the colonies lie near the surface, outgrowths are formed at the periphery which resemble hairs or locks of hair (35, I, i). The same completely surround the surface colonies (35, I, e). The colony then gives the impression of a ball of wool or tangled hair of a yellowish-gray color.

(*c*) *Magnified 150 times. Superficial colony:* The curly hairs appear as exceedingly long threads, which at the periphery lie singly, and toward the interior in parallel collections. These are regularly arranged like locks of hair (often intertwined like a whip-cord) (35, III). *Deep colonies:* The outgrowths of the deeply located colonies present coarsely granular, very irregular clumps, which are usually connected by means of nodular branches and fine processes. The colony does not present any center proper, but is very irregularly torn and exceedingly polymorphous.

Agar Stab.—From the stab canal, which remains white, there extend outward longer or shorter hairs, which become shorter as they are lower in the stab, and which terminate at times in curls or small clumps (34, VII). The surface growth is roundish, regularly spreading, with a smooth border, a little elevated, with a fatty luster, and gray, bluish, or yellowish-white in color. After a longer time there is often observed a formation of concentric zones (34, IX), or also, in other cases, of distinct, radiating folds passing outward from the center (34, VIII).

Agar Streak.—The growth remains limited to the inoculation streak; smooth edge, usually wavy. The color is grayish-white, somewhat transparent at the edge. The entire growth impresses one as if there were innumerable tiny, silvery air-bubbles lying beneath the surface. The water of condensation is clear or slightly turbid, with a little cloudy sediment (34, VI).

Bouillon Culture.—Homogeneous precipitate; bouillon clear, with most delicate, floating clouds. No pellicle is formed.

Milk Culture.—See page 307.

Potato Culture.—Rather dull, grayish-white or white, moderately elevated growth, limited to the inoculation streak. The border is wavy, sometimes notched. The growth stands out distinctly from the potato only when the latter is somewhat discolored. The appearance as of "silvery vesicles" is also observed here, as in the agar streak (35, VII).

Conditions of Spore-formation.—At temperatures above 12° there are formed in cultures which have the necessary supply of oxygen, oval, highly refracting spores. The higher the temperature (optimum 37°), the more rapidly the sporulation occurs; at the optimum, sporulation may be completed in eighteen to twenty hours. Günther gives the optimum at 28°; at higher temperatures the sporulation is not so regular. Weil obtained the most resisting spores at 37°.

Regarding the morphology of spore-formation, see page 26, etc. Plate 36, VI, shows the picture of fine bodies (spore antecedents) at regular intervals, occurring after four to eight hours at incubator temperature; Plate 36, III, represents mature unstained, and Plate 36, IV, mature stained spores.[1]

Regarding the germination of spores, see page 27.

Spores are never formed in the living animal nor in unopened cadavers (poverty of oxygen); on the contrary, they are formed upon anthrax meat after cutting it up, in bloody dejecta, etc. Weil has also observed anaerobic sporulation and a germination of spores without oxygen upon pieces of potato, quince juice, etc. (A. H. xxxv, 355).

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[1] Chauveau and Phisalix (Comp. rend., 1895, 801) have described a Forma claviformis, which sporulates like the Bact. tetani. Since we are here dealing with an absolutely non-virulent form, cultivated only in fluids, and not studied as to its morphologic properties upon solid nutrient media, it appears to us that the possibility of a substitution through a contamination is not excluded, even though previous treatment with this organism prolongs life a little in animals after the introduction of virulent anthrax. The observation deserves much attention.

Upon fresh nutrient media spores germinate in a few hours.

Cultures which are not transferred for a long time often lose spontaneously the ability to form spores. The bacillus may be deprived of its ability to form spores by cultivation upon nutrient media containing carbolic acid, or, with more difficulty, upon media to which are added bichromate or hydrochloric acid. Different cultures vary much as to the ease with which they become asporogenous. All agencies which reduce the virulence also operate unfavorably upon the sporogenous function, yet these properties are not necessarily associated; there are virulent asporogenous and absolutely non-virulent sporogenous varieties. Phisalix found in long cultivation at 42°, with frequent reinoculation, that the ability to form spores at 42° was gradually lost, but later the bacilli were also unable to produce spores at 30°. While at first the sporogenous function was recovered by inoculation of a mouse, after 14 reinoculations at 42° the sporogenous function was finally completely lost. The remaining virulence, still present at that time, after the twentieth generation at 42° was also lost (C. B. XIII, 533).

Viability and Resistant Properties of the Bacilli without Spores (Compare Momont, A. P., 1892, 21).—(*a*) In cultures the B. anthracis maintains itself (through spore-formation!) for many months.

In water: In an inhabited aquarium Höber found it dead in three to four days.

In soil: Moist anthrax blood is rendered free of germs in twelve to fourteen hours by sunlight.

(*b*) Drying: According to Koch, they remain alive, when dry, for five weeks at most; also in large dried pieces of meat they die in a few weeks. Bacilli in dried blood endure 92° for one and one-half hours, are killed by light in vacuum in eleven hours, and with admission of oxygen in nine hours.

(*c*) Salting does not kill anthrax bacilli in ham in fourteen days, but does in six weeks (Peuch).

(*d*) Moist heat at 60° kills rapidly.

(*e*) Cold: With an outside temperature of from —1° to —24° (average of —10.4°) bacilli in agar cultures were dead in great part in twelve days and almost completely in twenty-four days. The few which remained alive produced cultures with lessened power of producing disease and of liquefying gelatin.

Vitality and Resistant Properties of the Spores.—Stored in a dry condition, the duration of life appears unlimited. Spores remained alive in different samples of water and earth (with different conditions as to moisture), in putrid spleen, and in sewer contents for one and one-quarter to two and three-quarter years (Sirena and Scagliosi, C. B. XVII, 318).

Regarding the varying resistance to heat, see page 52; to chemicals, see page 53; to light, see page 53. Mormont found the resistance to light very great: spores in water die in sunlight after forty-four hours; when dry, they endure sunlight well for one hundred hours with admission of air, and for one hundred and ten hours with exclusion of air. The most resistant spores were obtained by Weil at 37°.

Chemical Activities.—Only those mentioned in the introductory remarks (p. 307) are known. The acids formed are acetic and caproic. There is slight formation of H_2S, and none of indol. Specific toxins could not be obtained from cultures by most writers. Compare the most recent, entirely negative, critical and experimental study of Conradi (Z. H. XXXI, 286).

Distribution.—

(*a*) *Outside the body:* So far found only, and always in the form of spores, in places or on objects which were contaminated with anthrax blood, etc.; for example, barn floors where anthrax cadavers had been skinned (G. Frank), skins, wool, and hair of anthrax animals, brushes prepared from the same, etc. They have not been demonstrated in the water and soil of anthrax meadows.

(*b*) *In man connected with disease:* As the cause of cutaneous anthrax (malignant pustule), inhalation anthrax (rag-pickers' and wool-sorters' disease, in the majority of the cases), and intestinal anthrax. In the first form the bacilli are only at the affected place and in the lymphatics leading therefrom; in the other forms they are also found in the blood.

(*c*) *In animals:* Common disease in cattle and sheep, rare in horses (very rare in swine), which graze in anthrax meadows. The infection takes place with preponderating frequency through the intestines by means of spores.

Regarding the findings upon section see page 313.

Experimental Observations Regarding Pathogenic Effects.—Especially susceptible are guinea-pigs and rabbits; somewhat less, sheep and cattle; much less, horses. Rats, especially dark-colored ones, are often quite highly immune; white ones always succumb, at least to a manifold infection. Swine, dogs, hens, and pigeons enjoy a very considerable, and the adult animals not infrequently a complete, immunity. (Regarding the variation of the same, see p. 96.) Frogs are killed, when kept warm, by ordinary anthrax; or, without warming, by anthrax adapted to cold temperatures (Dieudonné) (p. 45). For the susceptible animals, every possible method of introduction of anthrax bacilli and spores is successful. Feeding spore-free bacilli is especially uncertain (gastric juice kills); subcutaneous, intravenous, intraperitoneal, especially respiratory introductions of bacilli or spores are successful. After subcutaneous inoculation the animals show no symptoms for several hours. Frank and Lubarsch found that in the inoculation of guinea-pigs with an anthrax culture, which killed in thirty-four hours after subcutaneous infection of the animal, the bacilli first appeared in the blood seventeen to twenty-two hours after the infection. The section of the infected animal usually presents the picture of a septicemia. Besides hemorrhagic edema of the subcutaneous tissue (especially in the region of the point of inoculation), effusion into the body cavities, and splenic tumor, there are no especially characteristic changes. The bacilli are found in the blood, local edema, and in all the organs, especially in the spleen, but in variable numbers.

Variations in the virulence of the B. anthracis have been especially carefully studied. The virulence in ordinary cultivation is not very easily nor very much reduced, yet it is very easily attenuated by heat, chemicals, etc., until no virulence remains. (See p. 94.) Tavel once observed an anthrax bacillus (originating from smoked ham) which killed mice only after many—as much as thirty-two—days, and yet a man was killed from eating this ham.

By inoculation of cows and sheep with cultures of little virulence there is obtained a slight, and by subsequent inoculation with more virulent cultures a pronounced, immunity (similar experiments fail in mice and guinea-pigs, but sometimes succeed in rabbits), which, indeed, does not protect against the deleterious effect of feeding large quantities of virulent spores (Koch), but has proved very successful practically in anthrax regions (Pasteur). Hankin has tried to produce an immunity in animals with the metabolic products of anthrax without positive results; the serum from sheep in which a very high active immunity has been produced possesses immunizing action only for sheep and not for rabbits (Sobernheim). The bactericidal power of the serum in vitro is not increased over the serum from normal animals. The serum does not cause agglutination. Sobernheim considers the injection of a mixture of immune sheep serum (16 c.c.) and attenuated bacilli ($\frac{1}{10}$ loopful) to be the most certain method of conferring a more lasting vaccination protection (Sobernheim, Z. H. xxv, 301; and xxxi, 89). Emmerich and Löw have observed very noticeable curative and immunizing results in rabbits from pyocyanase (p. 110).

Special Methods for Demonstration and Differential Diagnosis.—If the question, as is usual, is one of diagnosis in an infected man or animal, very often a good preparation of smeared blood stained by Gram's method gives valuable results. For the differential diagnosis ordinary agar plates are especially to be prepared, which, after seventeen to twenty-four hours in the incubator at 37°, present spores within threads. Also observations as to motility are useful, as also are sugar-agar shake cultures.

The Differential Diagnosis between the Varieties which most Come in Question may be Represented as Follows:

	Anthrax.	Symptomatic Anthrax.	Malignant Edema.	Bact. vulgare (Proteus).	Bact. coli.	Streptococci.
Motility . .	0	+	+	+	+	0
Gram's stain	Very good.	Often good.	Usually negative.	Good.	0	Good.
Growth . . .	Aerobic.	Anaerobic.	Anaerobic.	Facultative anaerobic.		
Formation of locks . .	Good.	0	0	0	0	0
Formation of threads . .	Good.	0	Sometimes.	+	+	0
Fermentation of sugar . .	0	+	+	+	+	0 and +
Spores . . .	+	+	+	0	0	0

The result is always obtained with absolute certainty in thirty-six hours.

For the differential diagnosis between symptomatic anthrax and malignant edema, see page 314.

It may be difficult to differentiate an anthrax bacillus from soil from the closely related spore-forming varieties. If a virulent form is in question, then the inoculation with a sample of earth into several guinea-pigs will often decide the question. The dead animals will be examined as described above. Thus it is possible that one animal dies of anthrax, others of malignant edema, tetanus, etc., the causes of which were all present as spores in the sample of soil. Non-virulent forms of anthrax, isolated from soil, are only recognizable by comparison with known anthrax, whereby the five species of the table (p. 314) are to be excluded.

The following are described as closely related to anthrax:

B. pseudanthracis Burri. According to Hartleb and Stutzer, it is widely distributed in American meat powder. The cultures isolated from different samples were not exactly identical. The cultures are motile, especially when grown in bouillon. Also it is important that in bouillon there is first diffuse turbidity, then a clearing up, with the formation of a precipitate and pellicle. All other characteristics are deceptively like those of anthrax. Its virulence for mice and guinea-pigs is slight. Compare C. B. L. III, 81, where also is a description of cultures of B. pseudanthracis II and III, which are still a little further removed from anthrax.

B. anthracoides Hüppe and Wood (from soil), which we obtained from Král and carefully studied. We found, indeed, the agar cultures macroscopically very much like anthrax, but microscopically they resemble B. subtilis; also upon gelatin the similarity to Bac. subtilis when magnified 60 times was much greater than to Bac. anthracis. In young colonies loop-like projections extend out, reminding one of Bact. vulgare. When magnified 1000 times, sluggish motion was unmistakable.

B. anthraci similis McFarland (C. B. XXIV, 556). It was once found upon a laboratory plate, and is entirely non-pathogenic. Perhaps it was true anthrax.

Bacillus mycoides. Flügge.

(Plates 37 and 38, I–IV.)

Synonym.—Root bacillus.

Microscopic Appearance.—Rather large rods, scarcely at all rounded at the ends, 1.6–3.6 μ long and 0.8 μ thick. Sometimes arranged in threads (38, III). Forms oval spores.

Motility.—We have seen no cultures which exhibited active motility. Usually all the individuals are quiet except a few, which are only detected in motion after more prolonged observation. Also in the stained preparation the impression is given of only a few being provided with flagella. Formerly we believed we had observed entirely non-motile cultures, but can not now demonstrate this. Here belongs the non-motile **Bacillus radicosus** Zimm.

Staining Properties.—Stains by Gram's method.

Requirements as to Nutrient Media.—Slight; also grows without oxygen but scantily.

Gelatin Plate.—(*a*) *Natural size:* In earliest stage the colonies consist of a scarcely perceptible circle of little hairs (37, VI). After one or two days the gelatin is liquefied a little, while the colonies have become distinctly larger. The hairy circle ramifies more and more, and thicker branches are formed, especially at the center, which, toward the periphery, are replaced by irregular, fine, root-like branches (37, IX).

(*b*) *Magnified fifty times:* Colorless, more or less winding threads, interlacing in a most extraordinary manner. In the center the colony is sometimes felted and opaque. The branchings are only apparent, since always two closely lying threads diverge from each other at the point of apparent branching.

Gelatin Stab.—It is characterized by delicate little hairs of quite uniform length growing outward, parallel[1] to each other, along the stab canal (37, I). The liquefaction of the gelatin begins in the form of a saucer and then becomes cylindric. Upon the surface of the liquefied medium there is a thick white film, reminding one of a covering of asbestos. If this falls to the bottom of the liquid, a new one at once forms, so that cultures may be found having many such films.

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[1] In more advanced stages the hairs are often directed upward (37, II). The zone of liquefaction is usually clear or slightly cloudy.

Agar Plate.—(*a*) *Natural size:* At first the colonies are very like those in the gelatin plate, but more sturdy (37, VII). The further growth is absolutely irregular, there being found, as well, colonies with a dense center and very distinct main branches, and also those with a delicate central portion, and about this a growth in the form of a circle (37, VIII).

(*b*) *Magnified fifty times:* Exactly like the colonies in the gelatin plate. Plate 38, I, represents a colony with an open central part. Plate 38, IV, represents a part of the same magnified 150 times.

Agar Stab.—*Stab:* Parallel brush-shaped outgrowths, usually of unequal length, delicate gray, but a little denser than in the gelatin stab (37, IV). *Surface growth:* Exactly like the colonies on the agar plate; light gray, moist, shining (37, V).

Agar Streak.—Grayish-white, moist, shining growth, with root-like outgrowths, showing most extraordinary abundant branchings. In a short time it covers the entire surface (37, III).

Potato Culture.—Extremely like the potato culture of the Bac. subtilis. It is white, when older yellowish, a little elevated, crummy, dull, provided with a delicate, insignificant fringe at the periphery (38, II).

Chemical Activities.—See page 307. There is also no formation of H_2S.

Distribution.—Very common in soil.

Bacillus subtilis. F. Cohn. (Beiträge, Bd. i, H. ii, 175.)

(Plates 39 and 40.)

Common Name.—Hay bacillus.

Microscopic Appearance.—Short (1.3–3 μ), rather thick (0.8–1.2 μ), sturdy rods with rounded ends, often united in long strings, and not infrequently the separation into individual rods is indistinct, so that long threads occur (40, v).

Spores.—When air is admitted, oval spores are readily formed, which germinate at right angles to the long diameter. (Compare p. 27.)

Motility.—The short forms are very actively motile because of long, abundant, peritrichous flagella. The chains of bacilli still show flagella, even when they are no longer motile (40, VI, IX).

Staining Properties.—Stain by Gram's method.

Requirements as to Nutrient Media and Oxygen.—Grows upon the most various nutrient media at room and incubator temperatures, but, when oxygen is excluded it grows poorly and without sporulation. Growth is rapid.

Gelatin Plate.—(*a*) *Natural size:* In a short time the colonies sink into a saucer-shaped depression. The content of the liquefied zone is grayish-white. At the center lies the whitish, ragged colony (40, III). A later stage is shown in 40, IV.

(*b*) *Magnified sixty times:* At first the colonies are roundish, even-edged, crumbly, yellowish, sometimes surrounded by a delicate row of hairs (40, II, i). Later, especially in the case of the superficially located colonies, the periphery becomes wavy, and with advancing liquefaction of the gelatin breaks up into innumerable tangled locks, which surround the colony. The central part is still held firmly together, being granular and yellowish to brownish until after four to five days, when it also becomes completely disintegrated (40, II, e).

Gelatin Stab.—The surface growth is whitish-gray, and, after thirty-six hours, sinks into the gelatin in the form of a saucer. The gray liquefied content of the saucer contains whitish bunches in suspension (39, I). The liquefaction progresses in a cylindric form, the contents being grayish-white, and cloudy, especially below. Upon the surface is a thick white scum, firmly attached to the wall of the tube (39, II).

Agar Plate.—(*a*) *Natural size:* Small, irregular, shining, grayish-white colonies (39, VIII).

(*b*) *Magnified sixty times.* *Superficial:* Decidedly irregularly shaped colonies, rarely smooth-edged, usually extraordinarily ragged and fringed. The peripheral part consists of irregularly winding and interlacing threads, which at times may be rolled together in an impenetrable tangle. The center of the colony is yellowish, and finely granular. *Deep:* Similar to the superficial, but denser, thicker, and more opaque. The branches are still more irregular and gnarled (39, VII).

Agar Stab.—*Surface growth:* Moistly glistening, roundish, even-bordered, soon extending to the wall of the tube, a little elevated, dirty gray. Sometimes there is formed a pellicle or radiating wrinkles (39, V). (Compare also 34, VIII.) *Stab:* Thread-like or granular.

Agar Streak.—The growth upon the surface is like that upon the agar stab. The water of condensation is turbid, with a gray, cloudy precipitate (39, III).

Bouillon Culture.—Uniformly cloudy. There is pellicle formation upon the wall of the glass, and sometimes also upon the surface of the bouillon. There is a little whitish precipitate.

Potato Culture.—Dirty white to yellowish growth, with an undulatory, scalloped border, somewhat elevated, dull, never shining, spreading fairly widely, and upon long standing it has a meal-dust appearance (40, I).

Chemical Activities.—See remarks upon page 307. Charrin and de Nittis have obtained a pathogenic Bac. subtilis by cultivation upon nutrient media containing blood and by passage through animals. They always employed 0.5–0.75 c.c. of the most pathogenic culture to produce death in guinea-pigs. The affection remained local and the picture as a whole was more that of an intoxication. (Compt. rend. de la Soc. de Biol., 1897, 713.)

Distribution.—In hay, and widely distributed in soil. Besides this, also other forms with spores are present in hay, so that various kinds may be obtained by the method formerly recommended for acquiring the hay bacillus (inoculation of a sterile nutrient solution with a small quantity of fluid containing spores obtained by boiling hay for a long time).

It is not known to be of any practical importance. It cannot be transformed into anthrax, as was held for a long time.

Closely Related Varieties.—Bacillus leptosporus L. Klein, **Bac. sessilis** L. Klein (C. B. vi, 377), and **Bacillus malariæ** Klebs (see Schiavuzzi in Cohn's Beiträgen zur Biol. der Pflanzen, v, p. 245), which certainly has nothing to do with malaria. Compare Golgi (C. B. v, 506). The following are also very closely related:

Bacillus tenuis (Duclaux.) L. and N.

Tyrothrix tenuis Duclaux. Microscopically and upon gelatin plates, gelatin stab, agar streak, milk, bouillon, etc., it cannot be differentiated from the Bacillus subtilis. There is no trace of gas-formation from dextrose. On the contrary, the growth upon potato resembles that of Bac. vulgatus. The growth is pale red, much elevated, with a sinuous boundary, and traversed by voluminous folds. The variety stands midway between Bac. subtilis and vulgatus. It is worthy of notice that it does not stain by Gram's method. We have not found a form which ferments sugar.

Most closely related, if not identical, is the **Bac. implexus** Zimmermann, which Zimmermann described as non-motile, and which we ourselves always found non-motile in frequently repeated examinations made for our first edition (1896). The same cultures now exhibit lively motility, which is absolutely unmistakable. Contamination is surely excluded. Compare Zierler (A. H. xxxiv, 192) and Lehmann (*l. c.*, 196). This observa-
on is of the greatest interest.

Bacillus bernensis. L. and N.

dinary Name.—Aroma-producing bacillus from Emmenthaler
Burri (C. B. L. III, 609). In the same place is also found
literature regarding organisms with the smell of cheese.
rods (1.5 μ), facultative anaerobe. Spores are formed if
gen is admitted, which are twice as long as thick. Motion slug-
rarely active. Gelatin plate cultures differ in appearance from
of the hay bacillus type; gelatin stab and agar cultures are
what like those of the hay bacillus. Potato cultures are moist
smooth, lusterless, and without wrinkles. Bouillon becomes
dy, with pellicle formation. Milk is coagulated in about twenty-
rs, the coagulum being later dissolved. After about forty-

eight hours there is a strong, genuine odor of Emmenthaler cheese; also this occurs upon sterilized casein, precipitated by rennet. There is never any gas formed from sugar.

Bacillus megatherium. (De Bary.) Vorles. über Bak., ii. Aufl., 1887.

(Plate 41.)

Microscopic Appearance.—Rods 1.6–5 μ long, 0.6–0.8 μ thick, the ends not rounded, often united in long strings (41, x). These dimensions are a certain demonstration that the organism becomes smaller after prolonged cultivation (forma depauperata); we obtained this culture from the hygienic institute in Berlin in 1888. De Bary represents the thickness at about 3 μ. (Compare Bac. oxalaticus, p. 323.)

Motility.—By means of many peritrichous flagella it is rather slowly motile (41, XI).

Staining properties and requirements as to nutrient media, etc., are the same as in the Bac. subtilis.

Gelatin Plate.—(*a*) *Natural size:* Like Bac. subtilis (41, III).

(*b*) *Magnified fifty times. Deep:* Grayish, transparent, more opaque toward the center, finely to coarsely granular, as if beset over the entire surface with most minute hairs (41, IV). If the colony lies upon the surface, the periphery supports a row of rather long, very fine, delicate hairs, while the middle zone is a little lighter. The point at the middle remains compact (41, V). It resembles Bac. subtilis and Bac. mesentericus.

Gelatin Stab.—Tube- or sack-shaped liquefaction takes place along the stab. The liquid is turbid; sometimes, especially later, with cloudy flocculi. Later the liquefaction becomes cylindric (41, I).

Agar Plate.—(*a*) *Natural size:* White to grayish-white, a little elevated, moistly shining disks (41, VI).

(*b*) *Magnified fifty times:* In the earliest stages the deep colonies possess hairy or corkscrew-shaped outgrowths (41, VII, i), while the superficial ones possess a delicate, extremely transparent zone (41, VII, e). The latter in time becomes opaque, coarsely crummy, yellowish-brown, usually provided with winding, anastomosing lines. The deep colonies later appear irregular in form, with smooth edges, opaque, usually with outgrowths at the periphery (41, VIII).

Agar stab and streak like those of the Bac. subtilis (41, II).

Bouillon. — Moderately cloudy, often with pellicle formation.

Potato Culture.—Very similar to that of the Bac. subtilis. The color is usually somewhat more yellow; the mealy appearance also occurs (41, IX).

Chemical Activities.—See remarks on page 307; no indol, much H_2S.

Distribution.—Found by De Bary upon decomposing cabbage leaves. The **Bac. quercifolius** Lehm. and Detjen, from sausage, described from this institute by Detjen (dissertation) in 1890, appears to be identical.

Remarks.—The separation of this variety from the subtilis is rather difficult; it lacks the marked pellicle formation upon the gelatin culture and the pronounced formation of curls upon the gelatin plate.

The following, described by Hüppe, is very closely related:

Bacillus butyricus. Hüppe. (Mit. G. A. ii.)

(Plate 38, V–VII a.)

Judging from our studies of a culture which we have cultivated for six years, it seems to stand midway between the Bac. megatherium and mesentericus. The thinness of the rods appears to be a result of prolonged cultivation. Slender rods, 1.2–4 μ long, only 0.3–0.5 μ thick (!), with ends moderately rounded. They are motile, because of numerous peritrichous flagella, and stain by Gram's method. Upon the gelatin plates there occur, as in the case of Bac. vulgatus, typhoid forms; still, they are usually much scalloped, and often have a crater-shaped depression at the center (38, VI). Later the crummy center enlarges at the expense of the outer transparent zone (38, VII) until finally the whole colony breaks up.

Upon the gelatin stab culture a pellicle is likewise to be found, only the Bac. butyr. liquefies a little more slowly. The agar plate culture is exactly like that of the Bac. mesentericus, perhaps a little more delicate; also the agar streak and stab, except that the brown color is absent. The potato culture, on the contrary, presents no meshwork, and is not distinguishable from the megatherium (38, V). Bouillon remains clear, a pellicle forming upon the surface. Milk is coagulated, but sometimes remains fluid. No gas or indol, but some H_2S is formed. According to Hüppe, it elaborates butyric acid from salts of lactic acid; also from milk-sugar when it is previously hydrated by other bacteria.

Bacillus gastrophilus. L. and N.

This name is applied to a sporulating, motile, aerobic bacillus, which coagulates milk with the formation of lactic acid. Recently it has been repeatedly cultivated by Kaufmann and Strauss from the human carcinomatous stomach. The organism grows poorly upon the ordinary nutrient media, best upon beer-wort agar and gelatin, upon which it forms fine threads (according to the description, resembling anthrax). Old cultures appear as if covered with fine dust (air hyphæ?).

Details, together with literature, are given by Sternberg (Wien. klin. Wochenschr., 1898, 744), who cultivated the organism from an incarcerated hernia, and naturally disputes its diagnostic significance in carcinoma.

Bacillus oxalaticus. Zopf.

This organism possesses a greater interest because Migula (A. K., I. Bd., p. 139) conducted valuable studies upon bacterial structure upon it. The culture which we obtained from Král consisted of rods, which, from their relatively small diameter (0.8–1.6 μ), differ much from the thick forms (2.5–4 μ) which Migula observed. This is apparently due to cultivation. Motility and flagella were always absent. Upon gelatin plates the colonies at first resemble those of Bact. coli; later they become crummy and settle into the gelatin with a wide zone of liquefaction. With longer growth they become like the subtilis in appearance. In the gelatin stab the liquefaction is funnel-shaped, later cylindric, the contents are turbid, and a scum forms on top. The agar streak culture is not distinguishable from that of anthrax. Upon potato is formed a pure white, dry, later moistly shining, elevated growth. Bouillon remains almost clear. For chemical activities, see page 307; no H_2S nor indol.

Bacillus vulgatus. (Flügge.) Migula.

(Plate 38, VIII, IX; and Plate 42.)

Synonym.—Bacillus mesentericus vulgatus Flügge.
Ordinary Name.—Potato bacillus.

Literature.—Vignal: Le bacille mesentericus vulgatus, Paris, 1889. Not accessible to us.

Microscopic Appearance. — Slender rods, ends scarcely at all rounded, 1.6–5.0 μ long, 0.8 μ thick, often united in threads. It readily forms roundish to oval spores (42, XI).

Motility.—It propels itself by means of numerous peritrichous flagella (42, XII).

Staining Properties.—Stains by Gram's method.

Requirements as to Nutrient Media, Oxygen, etc.—The same as Bac. subtilis; grows rapidly.

Gelatin Plate.—(*a*) *Natural size:* After one or two days the colonies sink into the gelatin, grayish-white, delicate, wrinkled films being formed, which, even later, after liquefaction of the entire plate, do not disintegrate (42, VII).

(*b*) *Magnified fifty times:* In the early stage the colonies, especially at the periphery, resemble the smallest typhoid colonies until they begin to sink into the gelatin. (Compare also 43, XI.) Soon this transparent zone changes into a crummy mass, which is coarsely granular internally and possesses lobular markings, while the peripheral portion presents lobules separating from each other. Finally the entire colony acquires the appearance of a distinctly moruloid, brown, loosely attached heap, resembling a panther skin (42, VIII and IX). Besides the forms just described, there are often forms furnishing a transition to those of the Bac. mesentericus.

Gelatin Stab.—Upon the surface, a grayish-white, ragged-edged growth with a fatty luster. Gradually from this there is developed a dense film, which sinks into the gelatin in the form of a cup. The liquefied zone is cloudy with a dirty grayish-white precipitate (42, I).

Agar Plate.—(*a*) *Natural size. Surface growth:* White to whitish-gray, moistly shining, even-bordered or slightly granular, a little elevated. *Deep:* Roundish to whetstone-shaped, white. Sometimes in older cultures there occur folded or rounded elevations (42, IV).

(*b*) *Magnified fifty times. Superficial:* Roundish, gray, homogeneous colonies, without markings, becoming opaque toward the center, transparent at the periphery, and beset by long, often tortuous, plaited hairs (42, VI). *Deep:* Roundish to whetstone-shaped, gray, homogeneous, opaque, sometimes also with a limited development of hairs (42, V).

Agar Streak.—Luxuriant growth, with a wavy scalloped border, grayish-white, with a fatty luster, especially after a longer time becoming covered with numerous, irregular, considerably elevated folds. Toward the edge it is more transparent. The water of condensation is clear, and upon the surface of the same a firm film is formed (42, II). This description answers for the agar stab (42, III).

Bouillon.—A little cloudy, upon the surface a firm, grayish-white film, which is not broken up by shaking.

Milk Culture.—Slimy coagulum formed; strong alkaline reaction. Sometimes no coagulation occurs.

Potato Culture.—Exceedingly variable. The typical form at any rate presents abundant tortuous and confused more or less padded elevations, rising and falling precipitously, not unlike intestinal coils (42, X). The color is partly whitish-gray, partly yellowish, yellow, or even rosy brown. The coils may also be widely padded (38, IX) or appear as thick, moistly glistening elevations (like Bact. coli) (38, VIII).

Chemical Activities.—See remarks on page 306. No indol is produced and little H_2S.

Distribution.—Common in soil, thus a frequent contamination of our potato cultures (potato bacillus!). Also found in the intestine and in sausage (Detjen, Serafini).

Practical importance is slight. In incompletely unsterilized milk it, like the related varieties, occasionally produces a gradual coagulation with strong alkaline reaction, and later a solution of the coagulum with production of bitter-tasting, injurious substances.

The ability of the bacillus to sometimes produce abundant quantities of a slimy carbohydrate, especially in feebly acid bread, through swelling of its membrane, at times becomes troublesome. J. Vogel (Z. H. XXVI, 398; there also the literature), who has carried out in Hamburg a special study upon the bacilli of viscid bread, found especially two varieties of bacilli concerned with it: **Bacillus mesentericus panis viscosi** II Vogel, which essentially completely corresponds with the bacillus mesentericus L. and N. (see below), and **B. m. p. viscosi** I, which is distinguished by lack of motility, and by the flat, primarily non-characteristic, smeary growth on potato, which later forms large, parallel folds. The resisting spores withstand a short baking process.

The **Bac. gummosus** Ritsert (C. B. XI, 730), obtained from gelatinous infusion of digitalis, seems to be related. See also Happ (C. B. XIV, 175). According to both authors, this organism is only able to elaborate this slimy material from cane-sugar and not from grape- or milk-sugar. Besides, there originate mannite, grape-sugar, lactic acid, butyric acid, and carbonic acid. The **carcinoma bacillus of Scheurlen** (C. B. III, 397) has also been shown to belong to this group, but has nothing to do with carcinoma.

Bacillus geniculatus (Duclaux). L. and N.

Tyrothrix geniculata Duclaux. The gelatin plates macroscopically resemble Bac. vulgatus. When magnified 60 times, they present an interesting exhibition. The colonies at first appear upon the gelatin with delicate scalloping, like typhoid; with advancing liquefaction the scallops are replaced by curls, which in irregularity may compare with those of anthrax; still later, the circle of locks falls away and the compact colony floats in the shallow liquefied area, surrounded by irregular disintegrating masses. Also in the growth upon potato and in other properties it resembles the Bac. vulgatus. We have seen nothing of the branches in gelatin as described by Winkler.

Bacillus mesentericus. (Flügge.) Lehm. and Neum.

(Plate 43.)

Synonym.—Bacillus mesentericus fuscus Flügge. (Flügge, 3d Edit., p. 199.)

Microscopic Appearance.—Slender rods with rounded ends, 0.8–2.4 μ long, 0.7–0.9 thick, μ having a tendency to form roundish spores.

Motility, staining properties, and conditions of life are like those in the case of the Bac. vulgatus.

Gelatin Plate.—

(*a*) *Natural size:* Minute, roundish, grayish-white colonies, which very soon sink into the gelatin. The zone of liquefaction is flat, gray, cloudy. The colonies resemble very much those of subtilis (43, X).

(*b*) *Magnified fifty times. Superficial:* In the earliest stages they resemble those of typhoid, as do those of the Bac. vulgatus (43, XI). (See also 16, VIII.) With the onset of liquefaction the transparent peripheral zone becomes delicately granular, at the periphery appears a row of fine hairs, and the entire colony assumes the character of a liquefying subtilis colony. The center is usually grayish-brown and opaque (43, ix). *Deep:* Grayish-yellow, irregular; about the edge are curling, hairy outgrowths.

Gelatin Stab.—The colony after twelve to twenty-four hours sinks into a saucer-shaped depression. The liquefaction first assumes a funnel form and later progresses in a cylindric manner. The contents of the funnel are cloudy, with a whitish-gray film upon the surface (43, i).

Agar Plate.—

(*a*) *Natural size:* Roundish, gray, thin, veil-like growths, transparent, with the original whiter colony in the center (43, v).

(*b*) *Magnified fifty times:* The original colony lying beneath the superficial colony appears yellowish-brown, moderately or very crummy, with an even border or with curling outgrowth. When the colony reaches the surface, it forms a delicately punctated, transparent, irregular growth of a gray to yellowish color (43, vii).

Agar Streak.—Wavy, moistly shining, yellowish-brown, in many places gray and transparent. Water of condensation cloudy, with yellowish precipitate and a pellicle upon the surface (43, ii).

Bouillon.—Moderately cloudy, pellicle on the surface.

Potato.—At first the growth is moderately elevated, grayish-yellow, moistly shining, slimy (43, iii). Later it is transformed into a meshwork of irregularly anastomosing wrinkles, which are much elevated, of a yellowish-gray color, and possess a dull luster (43, iv).

Chemical Activities.—See preliminary remarks on page 306. It forms little indol and abundant H_2S.

Distribution, Practical Importance, Etc.—Like Bac. vulgatus.

Bacillus mesentericus ruber. Globig (Z. H. iii, 322).

Slender bacilli, 1 to 3.2 μ long, 0.4 μ thick, sometimes forming quite long threads. Not motile, stain by Gram's method, no spores. The gelatin plate exhibits quite variable forms. At first all the colonies present an appearance like typhoid; later some colonies retain the same, others form thick, moist, white growths, still others liquefy and form pellicles, and yet others resemble subtilis colonies. Upon the gelatin stab is formed a growth like typhoid, which still, after a longer time, slowly sinks in, with the form of a funnel. Potato cultures at first are like Bact. coli; later the cultures acquire a rose-color, which finally is transformed into reddish-brown. Agar stab culture is delicate, whitish-gray, transparent, moistly shining; later a netted film forms upon the surface. Bouillon becomes faintly cloudy, with a thin pellicle upon the surface. Milk is not coagulated, reaction feebly alkaline. H_2S and gas are not produced.

Bacillus aterrimus. (Biel.) Lehm. and Neum.

A very striking, aerobic, motile, sporulating bacillus, possessing all the peculiarities given on page 306, and producing black pigment. The gelatin plate cultures appear to resemble Bac. subtilis and vulgatus. Gelatin stab cultures present funnel-shaped liquefaction without coloration. Upon potatoes at first grayish-blue, then brownish-black, wrinkled, moist pellicles are formed, the potato being black throughout. Agar cultures become brown with yellowish-brown films. The organism is not pathogenic. Compare Biel (C. B. L. II, 137) and Lunt (*l. c.*, 572) regarding **B. mesentericus niger.** Gorini's closely related **Bac. lactis niger** (C. B. XX, 94) we obtained from Král in 1895 and studied. It no more showed any chromogenesis, and grew as a flat deposit upon potato, resembling the Bact. coli. Spontaneous motion could not be seen. It is questionable whether the insufficiently described **Bac. melanosporus** Eidam of Schröter belongs here.

Bacillus liodermos. (Flügge.) Lehm. and Neum.

Bacillus mesentericus liodermos Flügge.

This bacillus, described by Flügge as short and very actively motile, we have not certainly encountered during recent years. The gelatin growths in plates and in the stab are like Bac. vulgatus. The potato culture forms a smooth, shining, yellowish-white, syrupy growth, which only after several days becomes a little wrinkled and cloudy. The Bacillus mucosus Zimmermann (II, p. 8), from slimy water, appears to have some relationship.

Bacillus gangrænæ pulpæ. Arkövy.

Synonyms.—Bacillus fuscans Miller? Caries fungus of Galippe and Vignal, Caries fungus of Jung.

Literature.—Arkövy (C. B. XXIII, 917).

According to the investigations of Dr. Zierler, made in the Würzburg hygienic institute, which deviate somewhat from those of Arkövy in some points,[1] the following are the characteristics (compare Zierler, C. B. XXVI, 417):

Microscopically.—Stout rods, 4 μ long, about 0.8–1.0 μ thick, often united in chains. Actively motile. Staining of flagella has not been successful. Upon all nutrient media large, oval spores soon form, which are difficult to stain. Germination of the spores is equatorial, often oblique to the axis of the spore (Hirai). Grows luxuriantly on all nutrient media, best at 37°. *Gelatin plate:* Similar to subtilis, rapid liquefaction. *Gelatin stab:* After two or three days saucer-shaped liquefaction, which becomes cylindric. Upon the surface a very tough, wrinkled film, which usually remains connected by a fine string with the stab canal in the solid gelatin. The liquefied mass gradually becomes colored smoky brown. After two to four days fine, horizontal branches are seen throughout the entire length of the stab canal. *Anaerobic gelatin stab:* Without liquefaction and pellicle formation, the surface gradually becomes depressed. The branches in the stab are longer and more delicate. *Agar plates:* Thick plates exhibit superficial colonies something like subtilis (39, VI and VII); thin plates present dense, scalloped, concentrically striped, white growths, which, when magnified fifty times, appear opaque, and from the rather sharp border bunches of threads project outward. Deep colonies are compact and often whetstone-shaped. *Agar stab:* The growth rapidly spreads over the surface of the agar and is whitish, dense, with a dull luster; soon it becomes a little rough from the formation of small depressions and elevations. *Potato:* On first day, a delicate, membranous, moist, spreading growth; after three to four days the same becomes finely wrinkled and dirty red, and the neighborhood of the growth is stained dirty violet. *Bouillon* is very cloudy, with a dense, wrinkled surface pellicle. In a few days, with the formation of a rather crummy precipitate and brownish discoloration, the culture becomes clear. Neither H_2S nor indol is formed. Milk is coagulated after two or three days. In grape-sugar bouillon there is abundant formation of gas (principally CO_2) and moderate formation of acid.

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[1] Nothing was seen of the pleomorphism of Arkövy, and the "cocci forms" represented by Arkövy are often spores (C. B. XXIII; Plate XVIII, 10).

Arkövy always found the fungus in gangrenous teeth, and frequently in saliva and in gangrenous wounds (decubitus).

Zierler, like Arkövy, never failed to find the organism in gangrene of the pulp (24 cases examined), those in which the characteristic gangrenous odor was present. Not infrequently pure cultures were obtained, and, what appears especially remarkable, scarcely ever a culture of any other sporulating bacillus.

Zierler can not bring forward direct proof of the significance of the organism as the cause of gangrene. Arkövy has inoculated sound, broached human teeth with the bacillus and thus produced gangrene.

Further Sporulating Aerobic Varieties.

Here are included the thermophilic varieties which were spoken of from the biologic side in the general part. (See p. 44.) For the characteristics of the individual varieties we must refer to the original literature there cited, since they have only been partly named and are without great practical interest. They appear to be concerned in the spontaneous heating of hay, manure, etc., also in the hitherto puzzling bubbling fermentation. Laxa has described an organism which belongs here (C. B. L. IV, 362), and Poupé (C. B. L. IV, 484) another thermophilic, jelly-forming organism.

Introduction to the Special Description [1] of the Bac. tetani, Bac. Chauvœi, and Bac. œdematis maligni.

The three varieties have in common:

1. In pure cultures upon the usual nutrient media (agar, gelatin, potato) they are more or less perfectly anaerobic; on the contrary, they also grow very well

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[1] In the following, free use is made of v. Hibler's critical "Beiträge zur Kenntnis der durch anaërobe Spaltpilze erzeugten Infektionserkrankungen des Menschen, etc." Preliminary communication (C. B. XXV, 1899, 513, etc.). The detailed work has not yet appeared (June, '899).

aerobically upon boiled rabbit's blood, which before the inoculation is again briefly heated to 100° (especially true of Bac. tetani). The latter then forms spores excellently and its virulence increases (v. Hibler). It is also possible to grow anaerobes upon nutrient media containing sulphid of sodium, as stated on page 42. Regarding the behavior of anaerobes in aerobic mixed cultures, see page 43, and Kedrowski (Z. H. xx, 358).

2. Gelatin is liquefied, and (similarly to Bact. vulgare) fatty acids—from formic to caproic—and, besides, acids with aromatic groups (phenylpropionic acid, hydroparacumaric acid, and skatol-acetic acid), are produced (Nencki).

3. Also, without the sugar being present (!), according to Nencki, there arise from albumin: carbonic acid, hydrogen, H_2S, mercaptan, marsh-gas, perhaps free nitrogen (Bovet, C. B. VIII, 174). The gases have a very foul odor. Phosphoretted hydrogen, which smells like garlic and darkens silver nitrate paper, but not lead paper, in the latter differing from H_2S, has been found by Marpmann.

4. With the presence of sugar there originates a mixture of gases, with less of a putrid but with a sweet, repulsive odor, and consisting mostly of H_2S and CO_2.

5. Spontaneous motion produced by peritrichous flagella.

6. Spores are partly in the middle and partly polar. Attenuated as well as virulent cultures, when grown upon saccharine nutrient media, usually form spores only in the middle or incompletely in the pole, and of an oval form, which may be much elongated. Upon blood and blood-serum in all three varieties the spores are polar and round. In general, sugar and glycerin make the nutrient medium less favorable for spore-formation, and often it very soon fails to occur. This influence is more marked in symptomatic anthrax and malignant edema than in tetanus.

7. Regarding their resistance to injurious influences, consult the statements of Sanfelice. According to him, they were not nearly so resistant as the aerobic soil spores, being killed by 100° in live steam in fifteen minutes at most, sometimes also by 80°–90° rather quickly. It re-

mains an open question whether the spores employed in these investigations were of maximum resistance. Spores stored dry in earth remain alive for months, and even years; also, if the spores are placed in water together with soil, they live for months.

8. Upon rice nutrient media (rice covered over with a solution of 1% peptone and 0.5% NaCl) the virulence of all the varieties studied was soon lost (v. Hibler).

9. Most effective are intramuscular inoculations; less, subcutaneous; and least, intraperitoneal. The effect is more pronounced with extensive injury of tissue than without.

10. Attenuated cultures of the varieties producing local affections cause less edema and more cellular accumulation than virulent ones. The less the virulence, the greater the phagocytosis.

Bacillus tetani. (Nicolaier.) (Deutsch. med. Wochenschr., 1884, 842.)

(Plate 44.)

Literature.—Kitasato (Z. H. VII, 225; X, 267), Kitt (C. B. VII, 297), Knorr (Tetanusgift, Münch. med. Wochenschr., 1898, 321 and 362).

Microscopic Appearance.—In animal: rods, 1.2–3.6 μ long, 0.5–0.8 μ thick. In cultures (especially of little virulence) there are often very long threads,[1] sometimes also rods arranged in strings (44, IX). Mature spores are at the ends of the short rods, oval to round, 1.5–2.0 μ long and about 1.5 μ thick (44, VII). Many times a piece of a thread rests upon the end with the spore like a scepter. Sometimes also the long threads sporulate (44, X). Then in many places are seen short rods arising from the thread containing very distinctly polar spores; in other places one spore lies close to another, so that the entire substance of the rod appears to be converted into spores. Similar pictures appear to have been seen by Vincenzi (C. B. XIV, 149).

Spontaneous motion is observed in anaerobic hanging drops, dependent upon numerous, long, peritrichous flagella; according to Votteler (Z. H. XXVII, 480), 50 to 100 to a bacillus, which we have verified. According to Schwarz, there is only a single terminal flagellum!

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[1] Vincent and Kanthack claim to have observed branching.

Staining Properties.—Stains well by Gram's method.

Requirements as Regards Oxygen.—When freshly cultivated from the animal body (from wounds from which tetanus has originated, from nails, etc., which have caused tetanus), it is always an absolute anaerobe. After long cultivation in the stab (deep culture) the organism often gradually becomes less susceptible to oxygen. Cultivation is facilitated by the presence of certain saprophytes, which grow when oxygen is admitted. Recently Carbone and Perrero (C. B. XVIII, 193) have succeeded in cultivating virulent tetanus bacilli from the bronchial and tracheal mucus in a case of rheumatic tetanus, where absolutely no injury was to be found anywhere. The bacilli thrived much better and more luxuriantly aerobically, but in pure culture were no longer virulent. In the same place are also found references to the literature regarding earlier observations of aerobic tetanus cultures (Belfanti). Kamen (C. B. XVIII, 513) and Ferrán (C. B. XXIV, 28) made similar observations.

Intensity of Growth and Relations to Temperature.—Grows moderately rapidly, best at 36°–38°, and at 14° growth no longer occurs.

According to v. Hibler, the growths upon artificial nutrient media are the more luxuriant and sturdy, and the liquefaction of gelatin the more vigorous, the less pathogenic the organism is. Strongly pathogenic cultures often produce very slight growths. The findings of Tizzoni and Cattani have been similar.

Gelatin Plate.—(*a*) *Natural size:* At first minute, white, punctiform colonies, which, upon sinking into the medium, become surrounded with a transparent gray zone of liquefaction (44, IV; see also 45, VI).

(*b*) *Magnified sixty times:* Usually the colonies have a yellowish-brown, very crumbly center, from which there extends first a rim of short hairs; later innumerable, intertwining, interlacing, corkscrew-shaped threads. The older the colony, the more developed, longer, and more irregular these outgrowths become, and often they break up in a crumbly manner (44, III).

Gelatin Stab.—Deep in the gelatin along the stab there occurs first a cloudy growth, then cyst- or tube-shaped excavations, which are filled with cloudy, granular liquid (44, II).

Agar Plate.—(*a*) *Natural size:* Colonies whitish, roundish to ragged, usually surrounded by an exceedingly delicate veil (44, V).

(*b*) *Magnified sixty times:* The original colony appears grayish-yellow, roundish, opaque, surrounded by a broad zone made up of the finest interlacing hairs. Toward the periphery it becomes transparent; toward the center, grayish-yellow and opaque (44, VI).

Agar Stab.—In the stab prepared by a simple thrust of the platinum loop there develops, deep in the agar, a scaly, ribbon-shaped growth (compare 45, II). If one rotates the loop in the agar, then the growth extends in a wider zone and consists of a cone of cloudy layers (44, I), the surface of which, after a very long time, becomes covered with points and fine serrations (45, III).

Agar Streak.—No confluent growth, but only single discrete colonies (Votteler).

Blood-serum.—Sometimes liquefied, sometimes not.

Bouillon (anaerobic) moderately clouded.

Milk.—No (according to v. Hibler, very slow) coagulation; reaction amphoteric.

Non-albuminous Nutrient Media.—Upon Uschinsky's solution no distinct growth.

Resistant properties of the bacillus are without practical interest, as it sporulates very readily.

Resistance of the Spores.—See page 331, and Tizzoni and Cattani (C. B. IX, 487).

Chemical Activities.—See page 331. The forms studied by us form gas from sugar actively; a production of acid could not be demonstrated (on account of simultaneous abundant formation of alkali). Brain nutrient medium was darkened (v. Hibler). Extremely vigorous production of H_2S, little indol. Attenuated (slightly virulent) forms, according to Tizzoni and Cattani, often form more acid and grow more luxuriantly (C. B. XI, 150); general, the virulence is well preserved. Upon nutrient dia without sugar, we saw no gas production. The chemical activities of malignant edema and symptomatic anthrax are more vigorous. Regarding toxins, see page 74. The similarity of tetanus and strychnia poisoning, according to G. Brunner, is only superficial (C. B. XXIV, 629); on the contrary, Lusini even claimed that tetanus antitoxin had a favorable influence over strychnia poisoning (C. B. XXV, 325).

Distribution.—

(*a*) *Outside the body:* Wide-spread in garden soil, hay, and dust. Very often tetanus results in animals from the inoculation with samples of soil and dirt-floors from dwellings (Heinzelmann).

(*b*) *In healthy body:* In feces of horses and cattle, more rarely of man.

(*c*) *In man in cases of disease:* Cause of traumatic trismus and tetanus, puerperal tetanus, and tetanus neonatorum through wound infection. The organism is found only in the wound secretion, and usually in very small numbers; never in the blood and internal organs. "Rheumatic tetanus" appears (see above) to be due to tracheal infection with aerobic forms of tetanus.

(*d*) *In animals:* Tetanus often occurs spontaneously in horses; more rarely in sheep, goats, and other domestic animals.

Experimental Observations Regarding Pathogenic Effects.—

(*a*) *In animals:* The following are especially susceptible: Horses, guinea-pigs, goats, mice. Much less so: Rabbits, sheep. Dogs, rats (v. Hibler saw rats usually die), pigeons, and hens are almost immune, although the toxin remains quite long in the body of the hen. More details as to the immunity of the hen can be found given by Asakawa (C. B. XXIV, 166).

About twelve hours after subcutaneous infection at the root of the tail with virulent material, the mouse (similarly guinea-pigs and rabbits), which is the most commonly employed, shows the first symptoms of tetanus in a rigidity of the groups of muscles near the point of infection (tail, hind leg), and it goes about after the fashion of seals—*i. e.*, with the extended hind leg dragging upon the ground. Mild infection may cause unilateral tetanus and terminate in recovery. General exaltation of reflexes may be absent. In man and horses after subcutaneous infection the first symptoms are not local, but consist in a stiffness of special muscles—in man, the muscles of mastication; in horses, the muscles of mastication, membrana nictans, and those elevating the tail. Pure cultures cause no suppuration at the point of inoculation; the organisms remain limited to the site of inoculation and do not spread throughout the body.

According to Vaillard and Rouget (A. P. VII, 755), tetanus spores which are well washed, or freed from toxins by long heating to 80°, are harmless; trauma, metabolic products, admixture with other bacteria, protection of the spores by coverings, are necessary, in order that tetanus shall be produced. Other writers, as Roncali (C. B. XV, 439), dispute this; also, according to Dönitz, spores which were heated to 65° for one hour in 10% solution of chlorid of sodium retained their virulence (Deut. med. Wochenschr., 1897, No. 27, 428). The introduction of sterile tetanus toxin may kill animals with the symptoms of tetanus. A high degree of active immunity against tetanus may be produced by careful, repeated injections of sterile toxins, beginning with small doses and increasing gradually. By means of the serum thus obtained, which is rich in antitoxin, other animals may readily be passively immunized, and even small infected animals may be cured, but horses only with difficulty. A female mouse immunized against tetanus transmits a high degree of immunity to her offspring (for two to three months), but the immunized male does not. The milk of an animal immunized against tetanus preserves or produces immunity in the sucking young of herself or others.

(*b*) *In man:* Experimental infections of man with tetanus are lacking. Curative results from injection of tetanus antitoxin in cases of tetanus have often been claimed. For the older literature, see Remesoff and Fedoroff (C. B. XV, 115); for the latest condition of the question, see, for example, Erdheim (Wien. klin. Wochenschr., 1898, No. 19, 463), who, out of 22 new cases, reports 11 failures. (C. B. XXIV, 634.) Also in horses there re reported some favorable results and some failures.

Special Demonstration and Culture Methods.—The demonstration of the tetanus bacillus in the scanty secretion of wounds, usually cemented over, in tetanus patients may be difficult. In the first place, the wound secretion, which is scraped out, is examined in microscopic preparations for polar spores, whose demonstration under these circumstances may be looked upon as fairly certain proof of tetanus. In the second place, and it is never to be omitted, one inoculates a little of the secretion, but especially fragments and splinters of any foreign bodies found in the wound, into mice (p. 335), and finally an effort is made to cultivate the tetanus bacillus by means of anaerobic sugar-agar plates. Kitasato has recommended a preliminary heating for half an hour at 80° to get rid of spore-free, disturbing organisms; yet the virulence of tetanus spores is easily injured thereby. Heating to 60°–65° for ten minutes is sufficient to kill all spore-free contaminations.

Related Varieties.—Tavel (C. B. XXIII, 538) has described as **Bacillus pseudotetanus** Tavel a bacillus very similar to the tetanus bacillus, which is sluggishly motile, presenting only 8–16 flagella (tetanus, 50–100!), and which is found in the human intestine. It is a strict anaerobe and is not pathogenic for animals. Tavel is inclined, with Roux, to ascribe to the organism a significance in the causation of appendicitis and peritonitis.

Zimmermann (I, 50) describes his **Bac. gracilis** Zim. as a non-motile, facultative anaerobic bacillus with polar spores, which grows upon plates like the Bac. tetani.

Bacillus botulinus. van Ermengem. (Z. H. XXVI, I.)

Further Literature.—Brieger and Kempner (C. B. XXII, 765), Marinesco, Kempner and Pollack (C. B. XXIV, 899), Kempner and Schepilewsky (Z. H. XXVII, 213).

Vigorous rods, 4–9 μ long, 0.9–1.2 μ thick, sluggishly motile because of 4 to 9 flagella. Does not stain by Gram's method. Spores are usually polar. Sporulation is not interfered with by the presence of sugar in the nutrient medium.

In sugar-gelatin plates the colonies, according to v. Ermengem, are at first characterized by a smooth edge, which later develops a row of prickles, and by being composed of rather coarse, refractive granules, which are in constant motion. Later the border becomes much notched and irregular. Gelatin is liquefied. Stab cultures are not characteristic. In grape-sugar gelatin the growth is more luxuriant and accompanied by more vigorous formation of gas and liquefaction of gelatin; in ordinary gelatin it is not characteristic. We cannot see any essential difference, upon plates, between malignant edema, symptomatic anthrax, tetanus, and Bac. botulinus.

The most important biologic properties are: while grape-sugar is fermented very intensely, with accompanying gas-formation, milk and cane-sugar are scarcely affected. Milk is not coagulated. A marked foul odor never occurs even upon nutrient media containing no sugar, but only a sour, rancid odor. Sugar-bouillon becomes uniformly turbid, with a strong odor of butyric acid.

Optimum temperature, below 35°; obligate anaerobe. Growth is checked absolutely by more than 6% of chlorid of sodium. The spores are killed in half an hour by a temperature of 80°.

Pathogenic Properties.—The organism, when introduced by mouth or subcutaneously, produces the picture of botulism, without multiplying in the body. Filtered and devitalized cultures operate in the same way, since toxins are formed in the cultures. Brieger and Kempner have isolated the toxin, and have also obtained an antitoxin from the serum of animals which were poisoned for a long time. Especially susceptible to the poisoning when exhibited by mouth are guinea-pigs and mice; less, rabbits; still less, rats and pigeons; and least, cats, dogs, hens. Cats are also very susceptible to the subcutaneous introduction, but no local symptoms develop. The organism is the cause of certain cases of meat poisoning in which the enteric symptoms give place to nervous ones—dilatation of pupil, disturbances of accommodation, aphonia, paresis in the region of the tongue and pharynx (paralysis of swallowing), more rarely paresis of the extremities, and finally of the muscles of respiration. Together with this are present alterations of the salivary, bronchial, and pharyngeal mucous secretions (usually increased production), croupy cough, and interference with the evacuation of urine, bile, and feces. Consciousness is preserved. There is no fever. In a limited number of cases of poisoning by meat in Ellezelles in Belgium, the organism was cultivated from spores which were present in exceedingly poisonous ham, and also from the spleen of a man who had died after eating the ham. It appears to be rare, and could not be found by van Ermengem in the environs of men.

Bacillus Chauvœi. Aut. gallic.

(Plate 45.)

Synonyms.—Bacillus sarcemphysematis Kitt, Rauschbrand bacillus, Bacille du charbon symptomatique, Bacillus of symptomatic anthrax, Bac. anthracis symptomatici Kruse, Acetone or Forbicione of Italians.

Literature.—Kitasato (Z. H. VI, 105; VIII, 55); Ellenberger and Hofmeister, Path. der Haustiere (II, p. 458); Kitt (C. B. I, 684, 716, 741) (C. B. III, 572, connected review).

A detailed description of the cultures is unnecessary. As is shown in Plate 45, any sharp differentiation between this bacillus and the Bact. tetani by means of cultures is not possible, unless one makes much of the somewhat greater luxuriance of the cultures of symptomatic anthrax, as v. Hibler has shown. According to Votteler, the form of the outgrowths in the anaerobic culture upon slanted agar is more in round and arborescent lobules, not in rootlike outgrowths, as in malignant edema.

The bacilli themselves exhibit active motion, dependent upon peritrichous flagella (according to Votteler, 20 to 40, with which our results correspond), show a tendency to spindle forms upon nutrient media containing sugar, stain well by Gram's[1] method, and often possess spores which are a little nearer one end of the bacilli. Mature spores are typically polar upon serum (see p. 331). Besides, the bacilli contain easily staining, clear granules.

According to Kitasato, spore-formation occurs in the animal only after death. The viability of the organisms with spores in the dried flesh of symptomatic anthrax animals is very great. The chemical activities of the organism have been fairly completely investigated, most of the communications upon this being referred to upon page 331. Our cultures coagulate milk; v. Hibler found gradual coagulation. As the cause of symptomatic anthrax (**"Rauschbrand"**) (a destructive disease of cattle, localized in certain pastures, and formerly confused with anthrax) it is found in the bloody edema and muscles, the intestinal contents, and, what is of diagnostic value, always in the bile[1] of the affected animals. Cattle usually die in one and one-half to three days, with the development of a large crepitating swelling of the skin, regional swelling of the lymph-glands, high fever, and sopor. At the section there is found within the swelling a bloody, gelatinous edema, beset with gas bubbles, together with slight hemorrhagic exudate into the serous cavities, and peritonitis. The spleen is normal. The infection enters through injuries of the skin or mucous membranes. Of experimental animals, especially cows of one to three years (calves under six months less), goats, and sheep,[2] and most especially guinea-pigs and mice (rats somewhat less), are susceptible. Man is immune, also swine; dogs, cats, and abbits are rarely killed by it. In horses and kindred mals the inoculation produces only a local reaction. ective infections with attenuated cultures (or dried, rized symptomatic anthrax flesh heated to 100° for l hours) have proved very valuable. Immunization st symptomatic anthrax, according to Roux, protects gainst malignant edema; Kitasato found the oppo-

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[1] According to Günther, they do not stain by Gram's method.

so-called puerperal symptomatic anthrax, according l, is produced by the Bac. œd. maligni (C. B. XIX, Schneidemühl considers the Bacillus botulinus or a closely related organism to be the causative agent (C. B. XXIV, 577).

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[1] It is always necessary to examine not only the subcutaneous issue, but also the bile.

[2] The Norwegian sheep disease, Bradsot, is said to be symptomatic hrax (H. P. VII, 559).

Bacillus œdematis maligni. Koch.

(Plate 46.)

Synonyms.—Vibrion septique of the French. Bacillus of malignant edema.

Literature.—Koch (Mitt. a. d. Gesundheitsamt I, 53); Kitasato (Z. H. VI, 111); Jensen and Sand (Deut. Zeit. f. Tiermed., XIII); Penzo (C. B. X, 822); Horne (C. B. XIX, 77); Besson (A. P. IX, 179).

Microscopically. — Vigorous rods, like tetanus and symptomatic anthrax, but with a greater tendency (of diagnostic value!), especially in the cadaver, to grow into long threads. Active motion is due to rather numerous (20 to 40) peritrichous flagella, but it is only present in the short forms, the long threads being usually scarcely at all motile. In the short rods spores form at the middle sometimes, at the end sometimes, and they are oval or spherical. Our cultures were not stained by Gram's method, and most authors have obtained the same result. In cultures we found the Bac. œdematis maligni to be indistinguishable from the bacillus of symptomatic anthrax, as shown in Plate 46; we have not placed more illustrations in this plate because they would be only repetitions of those of symptomatic anthrax. We found only that the growth was somewhat more scanty than that of symptomatic anthrax, and the vitality of the cultures of shorter duration. Of importance is the universally demonstrated inability to sour milk (to break up milk-sugar); the milk is coagulated, with amphoteric reaction. Abundant formation of alkali by the organism reveals itself by darkening of the brain nutrient media. The chemical products have been much studied, and are essentially all enumerated on page 331. Also, in addition, in media containing grape-sugar there occur ethyl alcohol and inactive lactic acid (Kerry). A mixed culture of the Micr. acidi paralactici Nencki and the Bac. œd. maligni produces butyl alcohol abundantly, but neither variety alone can do it (Nencki, C. B. XI, 225).

Distribution.—Very widely distributed in soil, contaminated water, hay dust, etc. The inoculation of animals (best guinea-pigs) with samples of soil very readily produces malignant edema (still more often than tetanus). According to Horne, the most various septic diseases of domestic animals are occasionally produced by this bacillus. The section reveals, especially at the point of infection, a marked, bloody, gelatinous, often wide-spreading edema, with enlargement of the spleen.

Animal inoculations are made in practice by the subcutaneous injection of anaerobic bouillon cultures, most conveniently with not too small quantities of the edematous fluid from dead animals, or by the introduction of the infectious material into a deep cutaneous pocket. Spores without toxins infect with difficulty or not at all. The administration of toxins or the negatively chemotactic lactic acid increases the danger of infection very much (Besson, A. P. IX, 179). Also, according to Penzo, as in tetanus, the toxins formed in vitro are of the greatest importance in the outcome of the animal experiment; small doses of the pure culture he found to be without effect. Very small quantities of very virulent cultures suffice. Of the experimental animals, guinea-pigs and mice, and, in distinction to symptomatic anthrax, also rabbits, are very susceptible, and, besides, cattle, sheep, goats, horses, and pigeons.

The symptoms of the experimental disease in guinea-pigs correspond very beautifully with those in the spontaneous disease. Also in the case of animals dying from other causes, especially in warm places, bacilli may be found in the blood (having wandered from the intestinal canal) which are identical with or very similar to those of malignant edema; therefore care must be taken in the consideration of cadavers which are not fresh!

In the blood of recently dead animals the bacilli are not usually found microscopically (but may usually be demonstrated by cultures without difficulty), and very soon after death they spread everywhere, especially in the form of long threads. In the mouse, which is especially susceptible, there is also marked multiplication in the blood.

Infection occurs especially readily if the wound is contused or, as is very often the case in natural infection, other bacteria, hardly injurious of themselves, are simultaneously inoculated; for example, Bact. vulgare or Bact. prodigiosum.

Brief Differential Diagnosis.

See the key, on page 306, for the differential diagnosis between Bac. œdematis maligni and Bac. Chauvœi. For completing the diagnosis the following are to be carried out:

1. Examination of a fresh preparation for motility.
2. Two smear preparations are made from the edematous fluid or muscle juice and stained with fuchsin and by Gram's method.
3. Experiments upon guinea-pigs, and the examination of the phlegmon as to gaseous contents, and the bile for bacilli contained in it.
4. Experiments upon rabbits, which often give negative results with the Bac. Chauvœi.

Related Varieties (Pseudo-edema Bacilli).

Bacilli have been described by numerous writers which also kill experimental animals, with the production of bloody and emphysematous edema, but which do not correspond exactly with the bacilli of malignant edema or symptomatic anthrax. Recently v. Hibler has devoted a special study to these varieties and found representatives of most of the forms described which deviate somewhat in individual properties.

All these forms differ from malignant edema in the absence of thread-formation, growing as short rods, like symptomatic anthrax, and in pairs, and no more distinguishable from one another than some of the water vibriones. The following will give some idea:

Aside from the absence of thread-formation, the following correspond very well with malignant edema: the **pseudo-edema bacillus** of Liborius (Z. H. I, 163) (according to Liborius, it has two spores in a single cell) and the **Bacillus emphysematis maligni** of Wicklein (Virchow's Archiv, cxxv, 75), while Kerry's **new patho-**

genic anaerobic bacillus (Oest. Z. f. Veter., v, H. 2 and 3) differs in the rapid fermentation of milk and absence of a dark color in brain nutrient media. Klein's **new bacillus of malignant edema** (C. B. x, 186), and also Sanfelice's **Bacillus pseudoœdematis maligni** (Ann. Inst. d'Igiene di Roma, I, 375), do not liquefy gelatin nor coagulate milk, and darken brain nutrient media intensely. Compare also what is said under Bac. sporogenes Klein. These findings naturally make the diagnosis of symptomatic anthrax much more difficult.

Bacillus phlegmonis emphysematosæ. E. Fränkel.[1]

Synonym.—Bacillus capsulatus aërogenes Welch?

Literature.—E. Fränkel (C. B. XII, 13; and monograph, Hamburg, 1893); P. Ernst (Virchow's Archiv, CXXXIII, 308); Welch and Nuttall (Johns Hopkins Hosp. Bulletin, July and Aug., 1892; Journal of Experimental Medicine, I, 5, 45); Dunham (Johns Hopkins Hosp. Bul., April, 1897).

We are very much in doubt whether the producers of "gas phlegmons," "foaming liver," "development of gas in the blood and internal organs," described under these names, are always the same organism, and in what way they are related to other anaerobes. At any rate, considering the variability of bacteria, their identity is possible. All the organisms appear to have the following properties in common: they are plump bacilli, which are occasionally arranged in long pseudothreads, which present no spontaneous motion, are stained well by Gram's method, and very rarely (best upon blood-serum) form spores, which are observed either at the pole or middle of the bacilli. Welch and Nuttall usually found a capsule, the German writers say nothing about it. Grape-sugar is very rapidly fermented.

In guinea-pigs emphysematous phlegmon is produced (E. Fränkel, Ernst), in which the tissues are often destroyed like tinder. The effect upon mice is variously given.

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[1] The name of Welch is a little older, but, in the first place, it is not formed upon the binomial plan, and, in the second place, it is liable to cause confusion, since Bacillus capsulatus has been used repeatedly and there is a bacillus (or bacterium) generally known as aërogenes.

Welch and Nuttall found, at least in their first cases, no pathogenic properties for animals, but observed marked formation of gas in an animal which was killed soon after the intravenous injection of 0.5 to 1 c.c. of the culture.

The organism has been rather infrequently isolated from men with abscesses containing gas, etc. Here also belongs the **Bac. cadaveris butyricus** Buday (C. B. XXIV, 369).

Occasionally there also occur gaseous phlegmons and similar diseases of internal organs, in which are found the Bact. coli alone or usually in combination with other varieties, but without any anaerobes being present. See Bunge (Fort. der Med., 1894, XII, 533).

Bacillus alvei. Chesire and Cheyne (Jour. Royal Microsc. Soc., 1885).

Synonyms: Bacillus of the foul-brood of bees (French, "Loque").

Microscopically: Straight, fairly sturdy rods (0.8 μ thick and 2.5 to 5 μ long), sluggishly motile, large spores in spindle-shaped bulgings. The spores show polar germination. Our culture did not sporulate. *Gelatin plate:* Colonies first round, then they become provided with peculiar sturdy outgrowths, resembling wisps of straw or tendrils and very tortuous. Similar appearances occur in the stab culture. Gelatin is liquefied. In the stab there are often only single liquefying colonies, which are surrounded by radially arranged liquefying outgrowths; often the picture looks like an ink blot surrounded by fine outshoots. Upon potato a yellowish, upon agar a white, growth. Milk is first slowly coagulated; later the coagulum is dissolved and the reaction is faintly acid. The organism is only a facultative anaerobe; our culture from Král grows also aerobically. Aerobic potato cultures are delicate. It produces neither indol nor H_2S, and no gas upon nutrient media containing sugar.

It is the cause of the characteristic disease of bees.

We are not familiar with the actively motile **Bacillus piscidicus agilis** N. Sieber, which was described as a facultative, anaerobic, short bacillus with spores and which exhibited marked pathogenic effect upon fish (C. B. XVII, 888). O. Wyss held it to be the Bact. vulgare, which it certainly approaches very closely. N. Sieber, in his reply to Wyss, makes no mention of spores. Z. H. XXVII, 143, and XXVIII, 159.

The Anaerobic Producers of Butyric Acid.

While medical men have studied the anaerobic varieties from the pathogenic standpoint, and only secondarily investigated the zymogenic activities of the varieties isolated by them for purposes of differential diagnosis, whereby typical production of butyric acid was demonstrated in a number of varieties, also the ferment technicians have studied the anaerobic varieties as to their ability to form butyric acid, and have considered their pathogenic functions only secondarily or not at all.

We have not been able to study this group more closely, and cannot refer to the extensive literature in the absence of any comprehensive reviews,[1] but look forward to the exhaustive publication by v. Hibler. A review was supplied by Baier in 1895 (C. B. L. I, 17).

The following was found in Flügge's laboratory to be a vigorous anaerobic producer of butyric acid.

Bacillus butyricus. Botkin. (Z. H. xi, 421.)

Microscopically: Rods 1 to 3 μ long, 0.5 μ thick, often in chains in fluid nutrient media. They are motile and stain by Gram's method. The spores are in the middle or at the end, about 1 μ thick and are formed only upon non-saccharine media. Obligate anaerobe. Optimum temperature is 37°. Upon sugar-agar the growth appears like that of the other described anaerobes. Upon sugar-gelatin the colonies possess a slightly undulating border, as if consisting of a mass of felted threads, without the formation of branches. Gelatin is rapidly liquefied. After fifteen hours the casein of milk is precipitated, and butyric acid is formed, with violent liberation of gas; later the coagulum is soon dissolved. Upon nutrient media containing starch the bacillus presents inclosures staining with iodin. Starch is converted into sugar, and this directly into butyric acid. Also butyric acid is produced from milk-sugar without an intermediate formation of lactic acid. The organism is widely distributed and supposed to be non-pathogenic. The following may perhaps appear as a pathogenic form of this organism.

Bacillus sporogenes. (Klein.) L. and N.

Bacillus enteritidis sporogenes Klein (C. B. XVIII, 737; XXII, 113, 577; XXV, 278).

This rather strictly anaerobic organism stands very close to the Bacillus butyricus Botkin (see above), especially as regards the appearance, staining properties

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[1] Regarding Clostridium butyricum Prazmowski, see also v. Hibler, c. Regarding Clostridium butyricum M. Gruber I and II, see C. B. 367.

(Gram's, blue with iodin), motility, and spore-formation. The flagella are located at the ends especially in little bunches; the motion is sluggish. Also, in the edema no long threads are formed. The growths possess no morphologic value. According to v. Hibler, gelatin is slowly liquefied. Brain nutrient media is very slowly darkened. Milk is very rapidly decomposed, with vigorous formation of butyric acid and gas.[1] According to Klein, no spores are formed in milk in fourteen days, but they readily develop upon serum. Spores withstand 100° for one hour.

Subcutaneous injection kills guinea-pigs in eighteen to forty-eight hours, with the development of a wide-spreading, foul-smelling edema, containing abundant bacilli, and gas. Sometimes there is injection of the intestine and peritonitis. The spleen is not enlarged, and usually contains only a few bacilli and no threads.

In man the taking of milk which contains these bacilli in abundance causes severe gastro-intestinal disturbances (enteritis). So far, such occurrences have been observed only in England.

According to Klein, the bacillus is widely distributed in milk, intestinal contents of children and in cases of diarrhea, in street dirt, sewage, horse manure, etc.

Diagnosis.—The differential diagnosis from symptomatic anthrax is decidedly more difficult than from malignant edema. Attention must be paid to the presence of bacteria in the bile in symptomatic anthrax.

Other writers have described other producers of butyric acid. We give here, according to Klecki (C. B. L. II, 286), a comparison of three of them with the Bacillus butyricus Botkin. The literature is found in the same place.

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[1] According to Klein, if spores are repeatedly transferred from sugar-gelatin to sugar-gelatin, the organism loses the ability to form gas abundantly in milk; the milk remains alkaline and has a foul odor. The bacillus forms long, sporulating threads instead of the short members of the typical form; the virulence is almost or entirely lost (C. B. XXII, 581). If there has been no mistake here, and that seems improbable, then these observations are of very great significance, since they show how characteristics upon which two species were formerly based occur as two forms of one culture.

Bacillus amylozyme. PERDRIX (1891).	**Bacillus butyricus.** BOTKIN (1892).	**Bacillus orthobutylicus.** GRIMBERT (1893).	**Bacillus saccharobutyricus.** VON KLECKI (1895).
0.5 μ thick ; 2–3 μ long.	0.5 μ thick; 1–3 μ long; in fluids may be even 10 μ long.	1.5 μ thick; 3–6 μ long.	0.7 μ thick; 5–7 μ long.
Gelatin unfavorable nutrient medium ; not liquefied.	Round or oval colonies with slightly wavy borders, which appear as if composed of a mass of matted threads. Gelatin liquefied. Casein is dissolved.		Oval colonies with sharp borders and granular structure. Gelatin not liquefied. Very slight effect upon the casein.
Motile.	Slightly but distinctly motile.	Motile.	Sluggishly motile.
Anaerobe.	Anaerobe.	Anaerobe.	Anaerobe.
Forms spores.	Spores in the middle, but at times in the ends. Spores oval.	2–3 spores formed in one rod.	Polar, oval spores.
It causes fermentation of glucose, cane-sugar, and milk-sugar, and from them forms butyric acid, acetic acid, CO_2, and H_2. It ferments starch, thus producing butyric acid, amyl alcohol, some ethyl alcohol, CO_2, H_2.	It causes fermentation of milk- and grape-sugar and starch, thus producing butyric acid, propionic acid, acetic acid, formic acid, lactic acid, succinic acid, butyl alcohol, some ethyl alcohol, CO_2, and H_2.	It ferments glycerin, mannite, glucose, invert-sugar, cane-sugar, maltose, milk-sugar, galactose, arabinose, starch, dextrin, inulin, thus producing butyl alcohol, some isobutyl alcohol, normal butyric acid, acetic acid, formic acid, CO_2, and H_2.	It ferments milk-sugar, producing butyric acid, formic acid, CO_2, and H_2. Besides butyric acid, there is also apparently produced a higher fatty acid (valerianic acid).

Schattenfroh and Grassberger (C. B. L. V, 209) announce the discovery in the Vienna Hygienic Institute of three further types[1] of anaerobic butyric acid organisms, which were found while the Bacillus butyricus Botkin was being sought for in market milk.

They could not certainly identify their organisms with the numerous anaerobic varieties from milk which Flügge has described very briefly and designated by numbers.

Special Sporulating Anaerobic Bacilli as Agents in the Ripening of Cheese.

The interminably disputed and difficult question as to the part played by bacteria in the ripening of cheese is still not satisfactorily solved.

It is questionable whether non-living milk ferments play a part, as is believed by Babcock and Russell (C. B. L. III, 615). It is certain that bacteria are concerned in the ripening of cheese, and very evidently quite different varieties in different cheeses.

Duclaux was certainly right when he said that sporulating varieties play an important rôle in the ripening of certain soft cheeses (Cantal, Backstein, Limburger).

Weigmann described as **Paraplectrum fœtidum** (C. B. L. IV, 820) an obligate anaerobe, very like the bacilli of tetanus and symptomatic anthrax morphologically, but not pathogenic, which produces a pronounced odor of cheese. It is usually associated with the similar facultative anaerobe **Clostridium licheniforme** Weigmann, which usually forms spores at the middle, with swelling of the cell, and, perhaps, by exhausting the oxygen, it prepares the soil for the Par. fœtidum. Further details are found in the original, which is accompanied by photographs. The work shows how much remains to be done in the field of differential diagnosis of anaerobic varieties, and also how insecure the genera paraplectrum and clostridium are. (See p. 125.)

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[1] All three form butyric acid, but no alcohol, accompanied by abundant liberation of gas, from milk-sugar, starch, or grape-sugar. On the contrary, they are without effect upon dextrorotatory lactic acid. None of the three peptonized the precipitated casein; two are motile, one is not.

On the other hand, in the case of cheeses of the character of Emmenthaler cheese especially, von Freudenreich (for the similar Cheddar cheese, Russel and Weinzierl, C. B. L. III, 456) has reached the conclusion that here the "producers of lactic acid"—*i. e.*, those related to the Bact. acid lactici, etc.—are essentially concerned in the ripening, and that aerobic and anaerobic varieties do not necessarily participate, because they were found in ripe Emmenthaler cheese in very limited numbers, together with abundant generators of lactic acid. The addition of various spores cultivated from milk scarcely influenced the ripening process (v. Freudenreich, C. B. L. I, 168; V, 241).

According to Orla Jensen, the normal cavities in Emmenthaler cheese are due to the activity of the lactic acid bacteria, and contain CO_2, which is obtained from albuminous bodies and does not originate from carbohydrates (C. B. L. IV, 217). According to v. Freudenreich, a number of acid-forming varieties gradually attack the casein if allowed to operate for a longer time, and alter the character of the ripening.

Further, Gorini found that several organisms of the subtilis group change sugar into lactic acid only at higher temperatures, and regularly peptonize only when oxygen is admitted (C. B. L. V, 44). Also Burri (see p. 320) has described the Bacillus bernensis, a facultative aerobic, sporulating organism from Emmenthaler cheese, which has a pronounced cheesy odor.

The question is still being actively discussed. Those interested will constantly find articles upon it in the second part of the Centralblatt für Bacteriologie. (Compare also, v. Klecki, C. B. L. II, 21.)

Olav Johann-Olsen says that in the Norwegian "gammelost"—a fine cheese with a flavor of "apple, lemon, and Camembert cheese"—there must be a cooperation of lactic acid bacteria, Chlamydomucor casei, and Penicillium aromaticum casei; often also the process is aided by Dematium and a tyrothrix (C. B. L. IV, 161).

Anaerobic Bacilli as Causes of Fermentation of Cellulose.

While van Senus designated, as the cause of fermentation of cellulose, an anaerobic variety (Bacillus amylobacter v. Tieghem, according to van Senus) which operates only in symbiosis with an aerobic variety, Omelianski (C. B. L. II, 358, and V, 433) isolated a thin, anaerobic bacillus, which is not turned blue by iodin and forms polar spores, and which alone in a nutrient saline solution with peptone very readily causes fermentation of cellulose, with resulting formation of considerable quantities of volatile fatty acids (among them, normal butyric acid), CO_2 and H_2. We might give a large number of organisms causing decomposition of cellulose. The literature is given by Herfeldt (C. B. L. I, 114).

As **Amylobacter navicula** Wehm., Wehmer has described a facultative anaerobic bacillus, when sporulating assuming a clostridium form, which is motile when young, is partially stained blue with iodin, dissolves cellulose, and plays an important rôle in the wet-rot of potatoes. Wehmer has not carried out a sharp separation of this variety from related ones (C. B. L. IV, 734). He here also describes a second sporulating variety, but gives it no name.

Anaerobic Bacilli in the Retting of Flax and Hemp.

According to Winogradski and Fribes, the retting of flax (isolation of the bast fibers by softening in water) depends upon an anaerobic bacillus with terminal spores, which breaks up the cementing material (calcium pectate), with the production of butyric acid. Also the retting of hemp is brought about by an anaerobic bacillus, but it presents central spores and a blue color after iodin.

Gerstner (A. K. I, p. 152) has collected numerous anaerobic, sporulating varieties in addition to these, and has attempted—a perfectly thankless task—to arrange them in a scheme according to the descriptions found in the literature.

III. FAMILY SPIRILLACEAE (MIGULA). SCREW BACTERIA.

(For family and genus diagnosis, see p. 125.)

We have adopted the improved definition of genus as originating from Löffler instead of those of Müller, Cohn, and Ehrenberg for vibrio and spirillum.[1]

1. Spirals rigid:

(*a*) With one (rarely two or three) polar flagellum; very rarely without flagella. **Vibrio.**

(*b*) With a polar bunch of flagella. **Spirillum.**

2. Spirals flexible: **Spirochæte.**

Other writers have retained the somewhat older (1889) classification of J. Schröter, which is as follows:

1. Cells, bent into more or less pronounced screw forms, rigid, in the vegetative form actively motile, forming endogenous spores. **Spirillum.**

2. Vegetative cells slightly bent, rigid, usually with half a turn (comma form), actively motile, with arthrospores. **Microspira.**

This seems to us to have no advantages, but, indeed, great disadvantages, since spores are entirely unknown in most spirilla, arthrospores in microspira are denied by most authors, and, besides, the name microspira has been used by no one for ten years.

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[1] It certainly does not appear possible to make a sharp separation of the genera vibrio and spirillum according to whether they are provided with one or several polar flagella, and thus there is furnished a new proof of the necessity of great caution in establishing classifications upon the number and arrangement of flagella. According to Günther, his Vibrio terrigenus has a flagellum on each end, and often bunches of flagella! Kutscher has found some bent forms which present horny outgrowths, forkings, etc. Since Zettnow (Z. H. XXIV, 72) has photographed beautiful bunches of flagella upon the outgrowths, one cannot conclude that here involution forms are being dealt with. Severin has made similar observations in the case of his Vibrio denitrificans (C. B. L. III, 504). Here, however, the formation of branching forms is not under consideration, but triradiate forms (resembling a uterus). Compare the remarks in connection with the actinomyces.

I. Vibrio. (F. O. Müller, emend. Löffler.)

Cells short, slightly bent, rigid, comma-shaped, sometimes united in screw-like forms, usually only one, exceptionally two, polar flagella. There are no endospores. According to Hüppe, arthrospores are formed.

Key to the Recognition of the Most Important Varieties.[1]

1. Motile without phosphorescence.

(*a*) Gelatin slowly liquefied. Nitroso-indol reaction. Young gelatin plate colonies coarsely granular.

- (*α*) Usually not pathogenic for pigeons. **Vibrio choleræ** (Koch) Buchner, page 353.
- (*β*) Very pathogenic for pigeons. **Vibrio Metschnikovii** Gamaleia, page 366.

(*b*) Gelatin rapidly liquefied. No nitroso-indol reaction. Young gelatin plate cultures finely granular, brownish-yellow. **Vibrio Proteus** Buchner, page 367.

(*c*) Gelatin not liquefied. **Vibrio terrigenus** Günther and **Vibrio tonsillaris** Stephens and Wood Smith (C. B. XIX, 929), page 371.

2. Motile with phosphorescence. **Vibrio albensis** Lehm. and Neum., page 370.

3. Non-motile. (Spirosoma Migula). **Vibrio nasalis** Weibel, **Vibrio lingualis** Weibel, pages 375, 376.

Vibrio choleræ[2] (Koch). Buchner.

(Plates 47–51.)

Synonym.—Spirillum choleræ Koch.

Common Names.—Comma bacillus, cholera bacillus, "Bacille virgule" of the French.

Literature.—Petri, der Cholerakurs, Berlin, 1893. It contains all bacteriologic literature up to 1893. Voges has collected critically 139 more recent works (C. B. XIX, 466).

Microscopic Appearance.—Bent rods (about 2 μ long, 0.4 μ thick), the ends not lying in the same plane. The bending is often slight, scarcely perceptible; at other times pronounced (51, I, III), so that they are almost in the form of a semicircle. By the adhering together of two vibriones there occur such forms as these: $\begin{smallmatrix}(\\(\end{smallmatrix}$ and $\begin{smallmatrix}(\\)\end{smallmatrix}$. Under unfavorable conditions of growth (lack of oxygen, lack of albumin, etc.) the vibriones grow into true screw forms, which often cannot be recognized as composed of separate vibriones. According to Cramer, under especially favorable conditions (soda bouillon in a thin layer) there occur especially short oval or cocci-like formations. In old cultures there are manifold involution forms (51, IV).

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[1] Because of the close relationship of the varieties, the brief statements in the key can only point toward a diagnosis, and not furnish a complete description.

[2] In the description illustrations of related varieties are also referred to, when similar pictures occur exceptionally in cholera.

Motility.—Very distinct, rapid, turning motion, dependent upon one, rarely two, long, terminal flagella which are somewhat spiral in form (51, II).

Staining Properties.—Stains with the ordinary anilin dyes, but not especially easily; not by Gram's method. Usually carbol-fuchsin diluted ten times is employed for staining, it being allowed to act for a few minutes when warm.

Relation to Oxygen.—Aerobically, and much more slowly anaerobically, it forms powerful toxins.

Intensity of Growth.—Optimum at 37°, but also very well at 22°. The lower limit of growth has been found to be 10°–12°, sometimes 8°.

Gelatin Plate.—At first small, yellowish-white to yellow, roundish colonies, which as early as twenty-four to thirty-six hours sink into the gelatin in holes, and later in saucer-shaped areas of liquefaction.

(*a*) *Natural size:* The rapidly enlarging zone of liquefaction at first remains clear (48, VI); later it becomes cloudy, and usually gray, from the colonies disintegrating more and more (48, VIII). In many cases after a longer time there are present in the liquefied zone concentric rings (48, IX), which increase from day to day (48, VII).

(*b*) *Magnified sixty times:* After sixteen to twenty-four hours the colonies are visible as minute, pale-yellowish, roundish, coarsely granular disks with more or less of a crumbly character at the border (49, I). Often at this stage a beautiful, intensely red reflex appears at the periphery of the colonies. The older the individual colonies become, the more the granular character increases, and a stage is soon reached where the colonies appear to consist entirely of most minute, strongly reflecting fragments, looking, according to Koch, as if covered with broken glass (49, II). This is the most characteristic stage. The liquefaction now rapidly advances. The peripheral parts of the colonies disintegrate more and more (49, III, V), the structure appears fragmented and very granular, and sometimes a hairy border is formed at the periphery (54, V) or a gray transparent zone (53, III), until finally the entire colony is broken up into single fragments and small portions (49, VIII). Sometimes also the colonies may persist as compact masses in the areas of liquefaction (49, IX), when they are dark yellow to brown (50, IV), and there even occur forms which have absolutely no resemblance to cholera (50, I, II, V). In general, the variability is extraordinarily great, as is sufficiently shown in the illustrations (49, IV, VII; 50, III; 53, V; 54, V, VI).

On one occasion in a gelatin plate of vibrio aquatilis irregularly formed secondary colonies, resembling those of the Bact. coli, were observed, and similar ones of the vibrio choleræ (53, VII) may also occur.

Gelatin Stab.—At first thread-like and not characteristic (47, I; 53, II; 54, I). After a short time—twenty-four to thirty-six hours—there occurs upon the surface of the gelatin a very small perforating depression, which soon extends further in the form of a large air-bubble (47, II). In the depth the liquefaction extends in the form of a flattened funnel until the wall of the tube is reached (47, III, IV). Later the liquefaction becomes cylindric. The area of liquefaction is sometimes cloudy (47, III), sometimes only filled with the finest fragments (47, IV). In the stab canal granular, yellowish-white masses are usually implanted. It has been demonstrated by many observers that freshly isolated cultures of cholera vibriones are able to liquefy gelatin more vigorously than old laboratory cultures; therefore one must guard against recognizing rapid liquefaction of gelatin as evidence against the diagnosis of cholera. (See p. 61.) Such liquefactions as shown in Plate 54, II, III; Plate 53, I, II; Plate 52, I, II, are very unusual, but do occur.

Agar Plate.—(*a*) *Natural size:* Roundish, light brownish to white growths, with a moist luster, smooth borders, a little elevated, transparent (47, VIII, IX), sometimes resembling the colon colonies. (Compare also 18, VIII.)

(b) *Magnified sixty times. Deep colonies:* Irregular roundish and whetstone-shaped, with smooth or slightly roughened borders, with delicate or medium-sized granules, and pale yellow (48, I, II, III, right). Only after standing a very long time do they become darker colored (48, V) or present a brown central point with gray or greenish zones (48, IV). *Superficial colonies:* Roundish, faintly yellowish, transparent, at first extremely finely punctated (48, I, II), later coarsely crumbly (48, III). The picture after twenty days is shown in Plate 48, IV.

Agar Stab.—*Stab:* Whitish-gray, not characteristic, thread-like; later rough (47, VI). *Surface growth:* At first light brownish-gray, with a moist luster, wavy, smooth border, a little elevated, and after a longer time becoming colored a yellowish-brown (47, VII). The agar streak corresponds to this (47, V).

Serum Culture.—Solidified blood-serum at incubator temperature is rapidly liquefied.

Bouillon.—At incubator temperature after ten to sixteen hours there is a diffuse cloudiness, very often with the formation of a distinct, more or less rigid or friable pellicle. In cultures freshly isolated from the body, pellicle formation may sometimes be entirely absent; when the reaction is strongly alkaline, the pellicle becomes thicker and firmer (Cramer). Sometimes we have met with very compact, wrinkled pellicles, but in a subsequent culture upon the same nutrient medium nothing striking was observed.

Milk.—Koch described the vibrio choleræ as having no particular effect upon milk. More recently many writers have isolated cholera vibriones from typical cases of cholera which coagulate milk. The formation of acid appears to most of the authors to be sufficient explanation of the coagulation; a rennet ferment has not been demonstrated. For details, see Schoffer (A. G. A. XI, 262).

Potato Culture.—Upon faintly acid potato there is no growth or it occurs only at incubator tempera- According to Krannhals (C. B. XIII, 33), there are acid potatoes which become alkaline after standing and then become a good nutrient medium. The acid reaction may be gotten rid of by washing the sterile pieces of potato in sterile 0.25% to 0.5% soda solution or 0.5% to 0.75% solution of sodium hydroxid until the fluid becomes yellowish. If inoculation is made after washing off the fluid, the cholera vibrio will surely grow; also 2%–3% sodium chlorid solution performs the same service, although the reaction of the potato remains acid. Upon potatoes impregnated with sodium salts the cholera vibrio grows at 20°, not only at 37°. (Voges, C. B. XIII, 543.) Upon ordinary potatoes not thus prepared the growth is as follows: At first a dirty white to yellow growth, scarcely at all elevated, with a moist luster, not sharply outlined from the surrounding medium (50, VI). After standing longer, the yellow color is transformed into a brownish-red, while the culture spreads over the whole potato (50, VII).

Nutrient Media More Rarely Employed.—In sterile eggs the cholera vibrio grows very well, and here many varieties (also when every contamination is excluded) form abundant H_2S, while others form little, and still others none. Thus the long contest regarding this is settled. (See Abel and Dräer, Z. H. XIX, 61.)

A solution of 1% peptone and 0.5% chlorid of sodium in water (peptone-water) is much employed, especially for the demonstration of the formation of pellicle and indol. (See p. 371 regarding preliminary culture.)

The cholera vibrio grows very well upon Uschinsky's nutrient medium; according to Voges, with pellicle formation; but indol is never formed in it.

Spore-formation.—The formation of arthrospores as described by Hüppe (compare illustration on p. 25) has been verified by most subsequent investigators at the most in a botanical sense, and it appears to have no practical significance as far as the resistance of the vibrio is concerned. Also, Friedrich could never observe germination of the "arthrospores."

Viability.—

(*a*) In the sick: The vibrios have usually disappeared from the intestinal contents of the sick after four to eight or ten days, rarely sixteen days; in rare cases living vibrios have been found after forty-seven days (Rommelaire).

(*b*) In cholera stools the vibrios are usually alive after one or two days; more rarely, twenty to thirty days; still more rarely, longer; in one instance they were alive for one hundred and twenty days. Very similar results obtain in the case of clothing which is kept moist.

(*c*) In cultures: The cholera vibrio belongs among the varieties which die out easily. According to Gottschlich and Weigang, the number of living individuals in agar streak cultures very rapidly diminishes (Z. H. XX, 376).

Yet living individuals are usually found in cultures three months old, still frequently in those six months old, and now and then in those one year of age, if only too extreme drying is avoided. Morphologically such cultures consist almost entirely of involution forms. (Compare 51, IV.) According to Hüppe, also arthrospores.

(*d*) In water: Very different results have been obtained by writers as regards the viability of cholera vibrios when introduced into unsterilized water, varying from one day to one year. Low temperature, exclusion of light, and the presence of salts favor preservation; now and then, also, an increase is undoubtedly demonstrable. Most often in well- and river-water death of the cholera vibrios is observed in three to eight days. For more details see Ficker (Z. H. XXIX, 1). According to Hankin, the water of many Indian rivers kills cholera vibriones very promptly; these waters are said to contain "certain volatile, acid substances."

(*e*) Upon foods, usually a few days; coffee, one hour; beer, one or two hours; red wine, ten minutes. For further details compare Uffelmann (Berl. klin. Wochenschr., 1892, 1209) and Friedrich (A. G. A. VIII, 87).

Resistance to:

(*a*) *Desiccation:* Some statements are found on page 41; the entire literature is given by Ficker. Uffelmann upholds and William contests the possibility that currents of wind occasionally may distribute living cholera vibriones in a partially dried state.

(*b*) *Moist heat:* Killed in ten minutes at 60°.

(*c*) The resistance to *cold* is given very differently by various authors. All German investigators found them to withstand even very low temperatures for a short time, but our winter cold (5°–10°) was found sufficient to destroy them, often even in three, always in eight, days (Renk, Uffelmann, etc.).

Others, especially Russian writers, found greater resistance. Thus, Kasansky claims that neither a short exposure to a temperature of 30°, nor the operation of four months of Russian winter and repeated freezing and thawing, completely destroys the cholera vibrio. Similar results were yielded by experiments with Vibrio Proteus, tyrogenes, etc. (C. B. XVII, 184).

(*d*) For the effects of *disinfecting agents* see Kasansky (C. B. XVII, 506). The resistance is slight; especially acids are poorly borne. Iodoform vapor injures the cholera vibrio more than the other vibrios (Buchner, Bujwid).

(e) According to investigations by Palermo, cholera vibrios in *bouillon* are robbed of their virulence, but not killed in three to four hours by sunlight, and in six to seven hours become non-motile.

Chemical Activities.—

(*a*) *Chromogenesis:* Slight upon potato only. For cholera-red reaction see below (54, IV).

(*b*) *Odoriferous and gustative substances:* The disagreeable odor of cholera bouillon cultures, which is difficult to describe, was pointed out by Laser as of diagnostic value, but it is not sufficiently specific.

(*c*) *Formation of gas and acids from carbohydrates:* Dextrorotatory lactic acid is formed in abundance from sugar (grape-, cane-, and milk-sugar) without perceptible production of gas (Kuprianow, A. H. XIX, 282). In 10 c.c. of litmus milk the cholera vibrio forms a blue pellicle on top, the following layer is red, the deepest part is decolorized (reduction); thus the formation of alkali is favored by the entrance of oxygen, and the fermentation of sugar and formation of acid by anaerobiosis (Hellin).

(*d*) *Production of ferments:* Besides bacteriotrypsin, some invertin; also, according to Sclavo, rennet ferment.

(*e*) H_2S: In peptone bouillon rather abundant. (See egg culture, p. 357.)

(*f*) *Phosphorescence:* According to the statements of Rumpel, two cholera cultures ("Oergel" and "Elwers") were photogenic. R. Pfeiffer assumes that there is here a mistake, and denies that these photogenic cultures belong to cholera, basing his conclusion upon his immunity reaction described below (p. 373). It is also considered by most authors—for example, Dunbar—to be a photogenic vibrio from water, etc., and not a cholera vibrio at all. But recently Weleminsky, in Hüppe's institute, has observed two cultures of cholera vibrios become photogenic after passage through the body of pigeons, which were not so previously (C. B. XVIII, 285).

(*g*) *Indol:* Usually abundant production of indol upon nutrient media containing albumin or peptone. According to the number introduced, sufficient indol for demonstration is formed in peptone-chlorid of sodium solution in three to six or nine to twelve hours. Since simultaneously, from the small amount of nitrate contained in the peptone and chlorid of sodium,[1] etc., some nitrate is produced (Petri), indol can be demonstrated by the addition of sulphuric acid alone: "cholera reaction of Dunham and Bujwid," nitroso-indol reaction of the authors. After keeping the culture longer the intensity of the reaction increases somewhat up to twenty-four or forty-eight hours; later the nitrite gradually decreases, and, in order to demonstrate the quantity of indol, which increases for some days, some nitrite solution must be added (p. 78), when a dark violet-red color is obtained. A large loopful of an old agar culture will carry sufficient indol into 10 c.c. of peptone water for demonstration. The indol reaction rarely fails. (See p. 372.)

(*h*) *Toxins:* Manifold poisons have been produced from cholera cultures, but all are much less poisonous than the original material. According to R. Pfeiffer, these poisons are to be conceived as secondary, altered products from the disturbing action of reagents. Much more powerful but qualitatively similarly acting poisons are obtained from the bodies of the vibrios by very careful killing of the pure culture upon agar with chloroform or by brief heating, but the filtrate of young cultures is not poisonous.[2] Three times the quantity (about 0.5 mg. agar culture) of the minimum fatal dose of living bacteria, after being killed, also kills a guinea-pig in sixteen to eighteen hours. By longer heating the toxicity rapidly decreases. The effects of all these poisons when injected intraperitoneally are exactly the same as those following the introduction of living vibrios into the peritoneum: rapidly developing algid stage, muscular weakness, sleep, falling of temperature to 30°, death in sixteen to eighteen hours. Yet it must be emphasized that various proteins (from Bact. prodigiosum, Bact. coli), when introduced into the peritoneal cavity of guinea-pigs, produce the same symptoms (Hüppe, Klein, and others); also Voges obtained similar results with papain. Regarding the theory of Emmerich and Tsuboi (Münch. med. Wochenschr., 1893, 473, 497), that cholera is a poisoning by nitrite originating in the intestine, see page 94.

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[1] If the peptone and sodium chlorid are absolutely free of nitrate, then a weak solution of nitrate must be added. According to Bleisch, 40 drops of a 0.08% solution of saltpeter to 100 of nutrient solution was the proper quantity. If the nutrient medium contains too much nitrate, too much nitrite is supplied and interferes with the nitroso-indol reaction.

[2] Metschnikoff, Roux, and Taurelli-Salimbeni have obtained by means of all sorts of devices, fluid cultures of highly virulent cholera organisms, the filtrates of which were very poisonous. With such toxins also cholera antitoxins can be produced. While Pfeiffer's antibacterial serum protects animals very well from intraperitoneal infection, it is entirely without effect against infection through the stomach, against which the antitoxic serum affords some protection (C. B. xx, 627).

Distribution.—

(*a*) *Outside the body:* Recently they have been found not infrequently in water (wells, tap-water, rivers, harbors, canals), which had been contaminated with dejecta from cholera cases, yet their presence is only valuable if the differential diagnosis from the "water bacteria resembling cholera" is carried out with great caution. (Compare p. 373, etc.)

(*b*) *In the healthy body:* Not infrequently, in times of cholera, cholera vibriones have been found in healthy persons without any pathologic symptoms ("Choleragesunde"). For example, Abel and Claussen, upon repeated examination, found cholera vibriones present at some time in 14 out of 17 healthy persons who were members of 7 families in which there were cases of cholera; in many, for as long as fourteen days. Negative days intervened between the ones when positive results were obtained. In Hamburg 28 such cases of "cholera in health" with absolutely normal feces were demonstrated.

(*c*) *In diseased human organism:* Found only in cases of cholera, and in no other disease. The principal location is in the intestinal contents, especially in the mucous flocculi of the rice-water stool. There the cholera vibrio is often in pure culture; usually at the height of the attack they are present in large numbers, and generally decrease after four to fourteen days. In fresh cholera cases the organism is not usually found in the organs, except in the intestinal glands, where sometimes the epithelial layer is broken through. In exceptional cases, however, both in man and experimental animals, the vibrios are also found in the internal organs, as lungs, liver, kidney, spleen, and most rarely in the heart's blood. The more virulent the organisms, the more they spread into the organs.

(*d*) *In animals:* Spontaneous cholera in animals caused by cholera vibrios is unknown. (Compare Vibrio Metschnikovii, p. 366.) Our domestic animals, etc., appear to be immune to cholera infection, as it occurs in natural ways. (See below.)

Experimental Observations Regarding Pathogenic Effects.—(*a*) *In animals:* According to Sabolotny (C. B. xv, 150), the Spermophilus guttatus, a rodent of southern Russia, dies after being fed cholera vibrios with symptoms and section findings resembling those of cholera. Positive results per os were also obtained by Metschnikoff in young rabbits, by Wiener in sucking kittens and young (five days' old) rabbits, and by Karlinski in young dogs. (See Wiener, C. B. XIX, 205, 595.) In adult guinea-pigs by the natural channels, only an approximation to the picture of a case of cholera can be produced. Usually, following Koch's method, 5 c.c. of a 5% solution of soda is first introduced into the stomach, and shortly afterward 10 c.c. of a cholera culture in bouillon; at the same time 1 c.c. of tincture of opium to each 200 gm. of body-weight is injected intraperitoneally to quiet the intestinal peristalsis. Death occurs in twenty-four to forty-eight hours, preceded by a falling of temperature and extreme prostration. The intestine is reddened and contains abundant fluid, rich in cholera vibrios. Other vibrios, Vibrio proteus, etc., produce similar but not so pronounced effects. It is easier to kill animals (rabbits, guinea-pigs) by the introduction of the organisms into the blood-vessels or serous cavities. Death in peritoneal infection occurs in twelve to sixteen hours, usually after a primary multiplication, from the action of absorbed toxins originating from the dead vibrios (R. Pfeiffer). In the peritoneum (and eventually in the blood and organs) of the dead animal, living vibriones are usually found only when the infection has been produced with very large quantities. Many other bacteria operate exactly the same. (See p. 360 regarding the poisons of cholera.) If an animal withstands a single intraperitoneal infection with a small dose of living vibriones, it becomes immune to larger doses, because the bactericidal power is heightened, but the animal is not really more resistant to cholera toxin than it was originally. See below concerning R. Pfeiffer's biologic cholera reaction. See also R. Pfeiffer (Z. H. XVI, 258), M. Gruber, and Wiener (A. H. XV, 241).

One principal difficulty in the animal investigation of cholera is the variable, easily reduced virulence of the cholera vibrio. Many methods are recommended to increase the virulence; for example, the anaerobic cultivation in hens' eggs (Hüppe), which is contested by Wesbrook (H. R., 1896, 241), also passage through pigeons (Gamaleia, Salus, etc.). W. Rindfleisch, however, insists that no example of the cholera vibrio can be cultivated which is distinctly pathogenic for pigeons when injected subcutaneously (Z. H. XXI, 247). "Young" cultures, upon which many writers place great value, are only apparently more virulent, because they contain many more living individuals than older ones (Gottschlich and Weigang, Z. H. XX, 376).

According to Blachstein, the virulence of cholera vibriones is entirely dependent upon the nutrient medium. It is said that a cholera culture which is no longer virulent may be rendered virulent by cultivating it as follows:

1. Two days in a 2% peptone solution, which contains besides only 0.5% disodium phosphate and is cleared up with a little ammonium citrate solution.
2. Nine days in a 2% peptone solution containing also 3% potassium nitrate.
3. One day upon the solution given in 1, with the addition to each 100 c.c. of 1 c.c. of a cold saturated solution of ammonium-ferrosulphuric acid.

(*b*) *In man:* In a considerable number of cases, following the example of v. Pettenkofer and Emmerich, previously healthy men, after swallowing small quantities of pure cultures of the cholera vibrio, have developed the symptoms of cholera of slight or medium severity. The persons on whom the experiments were conducted usually had previously taken some soda solution to counteract the acidity of the stomach. Several severe and one fatal case of "laboratory cholera" have been known to occur in men who were working with cholera vibrios. (See Reincke, C. B. XVII, 202.) According to R. Pfeiffer, cholera in man arises, after destruction of the epithelial lining of the intestinal canal, by the enormously multiplied vibrios and the accompanying intoxication and absorption of poisons from the dead vibrios. We cannot here enter into a discussion of the teachings of Buchner, Nencki, and Metschnikoff, that the immunity against cholera in many localities is always or often dependent upon the absence of a synergetic or upon the presence of an antagonistic micro-organism in the intestine of the host.

Immunity and Immunization. — Recovery from cholera or an artificial cholera infection is followed by a certain immunity. In the peritoneal cavity of such an immunized animal cholera vibrios become granular and die (p. 374). The serum of the animal contains agglutinin (p. 374). With the cholera immune serum no considerable passive immunity in other creatures can be obtained, the conditions being very similar to those in pest.

On the contrary, Haffkine has obtained very good results in India in the production of active immunity by means of devitalized cultures. Kolle (Deut. med. Wochenschr., 1897) has repeated the experiments in the institute for infectious diseases, and found them confirmed in so far that the serum of the experimental persons contained bactericidal substances after about five days, which were most abundant on the twentieth day, but could also be demonstrated after a year. Various materials were injected; for example, one-tenth of an agar culture suspended in bouillon and heated for one hour to 56°. Virulent cultures operate similarly to non-virulent ones. For two or three days there is quite a painful infiltration at the point of injection. For the entire literature regarding cholera immunity see Voges (C. B. XIX, 466).

Varieties and Variations of the Vibrio choleræ.

Since first D. Cunningham (C. B. IX, 763, also XXIII, 854) demonstrated a considerable variation in cholera vibrios which he cultivated from typical cases of cholera, many writers have described forms which in part deviate very much. We can here only mention a few of these experiences, and only those where it appears certain that vibrios from true cases of cholera were in question.

A series of forms have been accurately described and photographed by Friedrich (A. G. A. VIII, 87), yet they do not deviate very widely from the typical organism.

More interesting than the reports regarding varieties are the observations regarding variability:

For example, the experiments made by Claussen in v. Esmarch's institute are very instructive. Vibrios freshly isolated from cholera stools presented upon plates a tendency for the colony to disintegrate and exhibit a border as if eaten away. The nitroso-indol reaction was absent. A guinea-pig did not die after the injection of 1 c.c. of a bouillon culture. The stab cultures grew slowly and were not characteristic. After repeated transfers in bouillon, a guinea-pig died after the injection of 1 c.c. of the bouillon, and in the peritoneal exudate, and even in the blood, cholera vibrios were found which possessed all the characteristic peculiarities, including the nitroso-indol reaction (C. B. XVI, 325).

Vibrio romanus of Celli and Santori, isolated from numerous typical cases of cholera in Rome in 1893, was cultivated from the stool, was not pathogenic for animals, gave no indol reaction, did not coagulate milk, and at 37° grew neither in bouillon nor on agar. After being culvtiated for eight months it gave the indol reaction and grew at 37°, but was still almost perfectly non-pathogenic (C. B. XV, 789).

Bordoni-Uffreduzzi and Abba cultivated from a typical case of cholera a very rapidly liquefying, short vibrio, which grew atypically upon gelatin and formed a yellow growth upon potato. After being cultivated for nine months upon gelatin it was constantly like the cholera vibrio, both macroscopically and microscopically (C. B. XVI, 201).

The Varieties Most Closely Related to the Cholera Vibrio.

When the cholera vibrio was discovered, its peculiarities seemed so characteristic that its differentiation from other bacteria was thought to be easy. Since then there have been found in the environs of man first a few, then more, and finally such an immeasurable series of vibrios that for a long time they have no longer been designated by separate names. The richest results have been yielded by the methodic examination of certain rivers. Thus, Dunbar (Z. H. XXI, 295) has published an entire series of Elbe water vibrios isolated from the water of Hamburg. Abbot and Bergey have collected 110 cultures of vibrios from the American Schuylkill River, on whose banks no cholera has prevailed for a very long time. Part of these are very similar to the cholera vibrio, and correspond most closely to the Vibrio Metschnikovii (Journal of Experimental Medicine, II, 535).

A detailed repetition of these descriptions would be senseless[1]; the description of the individual forms which are known by names is not even of much value, but in a measure serves to demonstrate the difficulty of differentiating "varieties." We give again a short description of the varieties which were carefully studied for the first edition, and, in connection with the same, refer continually to our illustrations.

Vibrio Metschnikovii. Gamaleia.

Principal Literature.—Gamaleia (A. P., 1888, II, 482), R. Pfeiffer (Z. H. VII, 347).

It is the cause of a disease of fowls occurring in southern Russia with symptoms resembling those of chicken cholera. Since its original discovery it has been also found by R. Pfeiffer in the north harbor of Berlin, and once by Kutscher in the Lahn. (See also above.) In the affected animals the vibrios are found in the intestine, and almost always also in the blood (Vibrio septicæmia).

This exceedingly interesting micro-organism can not be distinguished from the Vibrio choleræ by any morphologic peculiarities, therefore we have not made any illustrations of it. The vibrios are often a little more sharply bent and shorter than those of cholera (51, v). The liquefaction of gelatin varies exactly as in the case of the Vibrio choleræ.

It yields the nitroso-indol reaction without the addition of nitrite, and, according to Kuprianow, forms levorotatory lactic acid from sugar (like the V. choleræ).

The Vibrio Metschnikovii is remarkable for being highly pathogenic for pigeons and young chickens. If a trace of the culture is inoculated by a prick in the breast muscles, it causes death with local and general symptoms like those in chicken cholera (p. 210), only the intestinal findings are more like those of cholera than in the latter, and the spleen is rather shrunken than enlarged. The organisms are present in quantity in the blood and in the edema at the necrotic point of inoculation.

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[1] The forms known before 1894 are found together: Dieudonné (C. B. XVI, 363) and Brix (Hyg. Rundschau, 1894, IV, 913).

According to the statements of Gamaleia, cholera vibrios behave similarly toward pigeons, but Pfeiffer could verify this only by using very large quantities of cultures. Weibel (A. H. xxi, 22), Salus (A. H. xix, 342), Wlajeff (C. B. xvii, 619), and others, on the contrary, obtain inoculation results similar to those of Gamaleia with cultures which are originally virulent or rendered so artificially. The possibility of immunizing pigeons with the Vibrio Met. against the Vibrio choleræ is advocated from many sources, and denied by R. Pfeiffer, who also finds a reason for considering the Vibrio Met. a separate organism in its refusal to give Pfeiffer's serum reaction. (See p. 373.)

Vibrio Proteus. (Finkler and Prior.) Buchner, A. H. iii, 1885, 361.

(Plate 52.)

Vibrio "Finkler and Prior" of authors; "Finkler."

Literature.—Finkler and Prior, Ergänzungshefte z. Centralblatt f. allg. Ges.-Pflege., Bd. I, 279; Koch (Z. H. xiv, 329).

Microscopic Appearance.—More or less bent rods; on an average, 2.4 μ long and 0.4–0.6 μ thick, usually a little thicker than the Vibrio choleræ (51, vi).

Gelatin Plates.—With the unaided eye it only differs from the Vibrio choleræ in more rapid liquefaction and in the formation of larger disks (52, iii). Magnified sixty times, the colonies are yellow, with almost smooth borders, only slightly and finely granular (colonies of the Vibrio choleræ are coarsely granular with finely pectinate or crumbly borders). The surface colonies usually sink in rapidly and present a darker peripheral zone, sometimes with a row of hairs (52, iv).

Gelatin Stab Culture.—Tube-shaped liquefaction along the stab, without formation of any air space, and with marked turbidity of the contents (52, i, ii).

Agar Plate.—A little more luxuriant growth than in the Vibrio choleræ (52, ix). When magnified sixty times, the colonies look like those of Bact. coli (52, vii and viii). (See also 18, vii; 12, iv.)

Chemical Activities.—Milk is coagulated, and later again liquefied; faint acid formation; no gas formed from grape-sugar; indol reaction faint and frequently absent; very little H_2S developed.

Distribution.—(*a*) *Outside the body:* Claimed to have been once found in surface water (Héricourt).

(*b*) *In body:* In the intestinal contents or dejecta of some healthy persons, of some cases of diarrhea, and of men suspected of having cholera. Since its discovery by Finkler, in 1884, in the evacuations of persons said to be suffering from cholera nostras which had been kept a long time, this organism has been found but very rarely.

Pathogenic Significance in Man.—It is not the cause of the so-called cholera nostras; at any rate, in the great majority of the cases. Since its discovery, although much sought for, it has scarcely once been found in cholera nostras.

In experimental animals it produces in general the same, nominally somewhat milder, disease symptoms as the cholera vibrio.

B. Fischer found, in a case of suspected cholera, the **Vibrio helcogenes** Fischer, which was pathogenic for animals and resembles the Vibrio Proteus (C. B. XIV, 73).

According to Chantemesse, the **Vibrio lissabonensis** is identical with or very closely related to the Vibrio Proteus. It was discovered by Pestana and Bettencourt (C. B. XVI, 401, photographs) in the spring of 1894 in numerous cases of an epidemic, widely distributed, mild, choleriform disease in Lisbon, and was also found in the city water aqueducts. It is a slightly bent vibrio with polar flagella, giving no nitroso-indol reaction, and without pellicle formation upon bouillon. It produces liquefaction of gelatin in the upper part of the stab culture in the form of a broad, flat funnel. In the gelatin plates there appear upon the surface colonies, which at first are round, smooth, and only slightly granular; later they have a gray center surrounded by a scarcely transparent, granular zone, which is limited externally by a thick circle of fine radiating threads of considerable length. Because of progressing liquefaction the characteristic appearance is lost by the third day. Upon ordinary potato it grows very poorly, but upon alkalinized potato very well as a shining gray growth. The organism is slightly pathogenic for animals. It does not immunize against cholera.

Vibrio tyrogenes. (Deneke.) Lehm. and Neum.

Synonyms.—Deneke's cheese spirillum; Spirillum tyrogenum Deneke (Deut. med. Wochen., 1885, 33).

Isolated by Deneke from an old cheese, but since then it has been very rarely found. As regards intensity of liquefaction, it stands midway between the Vibrio choleræ and Vibrio Proteus, and also in other respects its peculiarities are usually so intermediate between these two varieties that we have not illustrated them. The peculiarities mentioned by Günther (Bakteriologie, IV. Aufl., p. 361)—a thick mold-like scum upon the gelatin stab culture and a marked yellow color of the same—were not observed in our cultures. Our culture gives the nitroso-indol reaction like the Vibrio choleræ. According to Kuprianow, it forms dextrorotatory, and the Vibrio choleræ levorotatory, lactic acid. Our old laboratory culture grows well at 37°.

Vibrio danubicus. Heider. (C. B. xiv, 341.)

(Plate 53, I, III, IV.)

Nothing peculiar microscopically (53, IV). Gelatin is powerfully liquefied. Stab cultures remind one of very actively liquefying cholera cultures. In our cultures the form of liquefaction was always more like a saucer than a flattened funnel. Upon very thick plates it is very similar to the cholera vibrio; upon thinner plates, after twenty-two hours at 22°, the surface colonies spread out exceedingly thin, are irregular, and have a border which is wavy or provided with coarse outgrowths. They are almost colorless and very delicately and uniformly marked with fine striations. Our illustration corresponds with this in general (53, III). Milk is coagulated; upon potato there occurs in the incubator a brownish, miserable growth. It gives the indol reaction well. Pathogenic for guinea-pigs, less for pigeons. Cultivated by Heider from the water of the Vienna canal of the Danube at a time when no cholera was known to exist in Vienna; later detached cases of cholera occurred.

Vibrio aquatilis. Günther. (Deut. med. Woch., 1892, 1124.)

(Plate 53, II, VII, VIII, IX.)

Microscopically not specially different from the cholera vibrio (53, VIII). The colonies in gelatin plates, however, are easily distinguished from those of the cholera vibrio by the smooth or slightly wavy border (never with granular irregularities) and very fine granules (53, IX). In Plate 53, VII, we have reproduced a quarter of a very remarkable deep picture in a thinly sown gelatin plate. The surrounding, numerous, secondary colonies are to be explained by softening of the gelatin (too high temperature). Older gelatin plate cultures are similar to the cholera vibrio; the liquefaction is slow. There is no nitroso-indol reaction, but a strong odor of sulphuretted hydrogen. It is not pathogenic. Weibel found a similar vibrio in a well which had been infected with cholera vibrios a long time before (C. B. XIII, 117).

Vibrio berolinensis. Rubner. (Neisser, A. H. xix, 194.)

(Plate 53, V, VI.)

Microscopically like the Vibrio choleræ (53, VI). We also found the gelatin plate cultures very similar to those of cholera. There is a tendency to the formation of coarser lobulations, and a finer granulation of the colony is striking. Liquefaction of gelatin is minimal. Strong nitroso-indol reaction. Considerably pathogenic for guinea-pigs.

Vibrio albensis. Lehm. and Neum.

(Plate 54.)

Synonyms.—Phosphorescent Elbe vibrio of Kutscher, Dunbar.

A detailed description is unnecessary in the face of the fact that the best judges of the photogenic vibrios do not presume to differentiate them morphologically from those of cholera. Our cultures show very constantly—as they are usually described—luxuriant growth, vigorous liquefaction in the stab canal, pellicle formation on bouillon, and vigorous indol reaction. The gelatin plate colonies we were unable to certainly distinguish from cholera (54, VI). We often observed in old superficial gelatin plate colonies a pretty circle of hairs, as is presented by many vigorously liquefying varieties, but which we have never met with in the cholera vibrio. In the six cultures of photogenic Elbe vibrios obtained, the phosphorescence was vigorous, but often, through insufficiently frequent transfer to fresh nutrient media, it was completely lost, and in some experiments it could not be regained by employing herring nutrient medium. Marpmann refers the phosphorescence to the formation of phosphoretted hydrogen.

Judging from the descriptions, a number of photogenic inhabitants of the sea, described as bacilli or photobacteria, appear very closely related to the Vibrio albensis. We may place them here, naturally without expressing ourselves as to how far they are different "species."

Vibrio indicus (Beij.) Lehm. and Neum. Bacillus phosphorescens Fischer (non Bacterium phosphorescens Fischer, which is found on page 231). Photobacterium indicum Beijerinck (non Bacillus indicus Koch, which is found on page 274). West Indian photogenic bacillus. The gelatin plate and stab cultures are described as like cholera throughout; the liquefaction is intense. Microscopically: small rods, two or three times as long as thick, very often in pairs, more rarely threads. In chlorid of sodium milk, screw forms occur. Active serpentine motion. The light is bluish-white and intense. Minimum, 15°; optimum, 30°–35°; maximum not much higher. According to Beijerinck, it is also able to emit light upon non-saccharine nutrient media, but also does so with the addition of a little sugar.

Katz considers the **Bac. cyaneophosphorescens** Katz, obtained from Australian seas, to be closely related (C. B. IX, 156[1]). According to Katz, however, this organism occurs as straight motile rods and curved non-motile threads.

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[1] In the same place Katz has also described completely four other "varieties": **Bacillus argenteophosphorescens I, II, III, and arg.-phosphorescens liquefaciens.** They appear in part to be also vibriones.

Vibrio luminosus (Beij.) L. and N. (Photobacterium luminosum Beijer.), obtained from the North Sea. It is very closely related to the Vibrio indicus, according to Beijerinck. It liquefies vigorously and presents vibrios and spirilla. According to Beijerinck, it also is photogenic without the addition of sugar. Slight addition of sugar favors photogenesis; a little more (1% or more of dextrose) inhibits it.

Vibrio balticus (Beij.) L. and N. (Phot. balticum Beijer., C. B. VIII, 616). "Native phosphorescent bacillus" Fischer (C. B. III, 105), from the Baltic Sea. Described by Fischer as very similar to the Vibrio indicus. Light, bluish-white. In the description of the microscopic character and the appearance of the cultures, Fischer himself often compares it to the Vibrio choleræ. Minimum, below 5°. It produces light, according to Beijerinck, only upon nutrient media which contain sugar. It bears very well a large proportion of sugar (3%-5% of cane-sugar). The freshly isolated cultures liquefied very little. Beijerinck finally obtained very vigorously liquefying cultures by longer cultivation on gelatin. It does not ferment sugar.

Vibrio Fischeri (Beij.) L. and N. (Photob. Fischeri, Beijerinck; C. B. VIII, 616). According to Fischer, it stands very close to the Vibrio balticus. When freshly isolated, it liquefied very vigorously, and gradually almost completely lost this property. Traces of cane-sugar favor the photogenesis; 0.5% or more lessens it. It does not ferment sugar.

Vibrio terrigenus. Günther (C. B. xvi, 746).

Does not liquefy gelatin at all, forms a delicate pellicle upon gelatin. It is interesting, from the standpoint of classification, that it possesses either a single flagellum or a bunch of flagella at each end. Gelatin colonies are smooth-edged and structureless; the superficial ones form little heaps. Older deep colonies are brownish and studded. It produces a good yellowish-white growth upon potato. Sugar is not fermented, milk not coagulated. It is not pathogenic for animals, and is an obligate aerobe. Obtained from Berlin soil. The **Vibrio saprophiles** α, β, γ Weibel appear to be similar (C. B. IV, 225, 257, 289).

Special Methods for the Demonstration of the Cholera Vibrio.

The examination should usually be completed in twenty-four to thirty-six hours.

A. In the evacuations of cases of cholera or suspected cholera.[1]

1. Microscopic preparation (usually from a flake of mucus !): The presence of abundant vibrios (especially if arranged parallelly like

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[1] The demonstration is conducted in the same manner in the case of milk and other foods, soiled linen, old dried laboratory cultures, etc. Here often the direct microscopic observation can be omitted.

a school of fish, according to Koch) speaks strongly in favor of cholera, for vibrios resembling those of cholera, if present in the stools at all, are usually only scanty. If the stool is of nearly normal consistency, the direct microscopic examination may be omitted. One should avoid mistaking the thin spirilla (Sp. hachaizæ) for vibrios.

2. Testing of a fresh, minimal specimen of the stool which contains living vibrios in great number with serum, as on page 373.

3. Infection of an alkaline peptone chlorid of sodium solution [1] (about 50 c.c.) with a flake of mucus or with 1 to 5 c.c. of the stool. This is to be kept at incubator temperature. **(Preliminary cholera culture.)**

(*a*) Observation of the pellicle formation. After three hours indication of pellicle formation may be present. After about sixteen to twenty-four hours the pellicle does not become more distinct. (Many micro-organisms form pellicles!)

(*b*) Microscopic demonstration of vibrios in pellicles. Here the occurrence of vibrios demonstrates much less the presence of true cholera vibrios than does a large number in the stool. Also vibrios resembling cholera may develop into pellicles.

(*c*) Agar plates from the pellicle (37°) after eighteen hours must not be phosphorescent.

(*d*) Gelatin plates from the pellicle (22°). After sixteen to twenty-four hours, when magnified sixty times, the characteristic shining and coarsely granular colonies are found. The form of the growth in gelatin is one of the principal characteristics. The suspicious colonies (if not numerous, all are considered) are inoculated as soon as practicable into gelatin (flattened funnel-shaped liquefaction) and tubes of peptone chlorid of sodium solution (indol reaction).

(*e*) Indol reaction (without nitrite being added) with part of the tubes after three hours. The indol reaction is usually certainly present in cholera after eighteen hours. By rapid transformation of the nitrite into ammonia, various water bacteria can frustrate the direct cholera reaction. See page 359 regarding the failure of the indol reaction in pure cultures of certain cholera.

(*f*) Potato cultures from the pellicle. Chlorid of sodium potato (p. 357) at 37°. Yellowish-brown to brownish-red color is in favor of cholera.

4. Gelatin plates prepared directly from the stool (3 dilutions). Abundant colonies of vibrios with a form like those of cholera speak very strongly for cholera even if the liquefaction appears too vigorous.

5. Agar plates smeared over very thinly with very much diluted stool and kept at 37°. Photogenic colonies are not looked upon as cholera.

6. All vibrios isolated in these ways must be examined with the

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[1] For the preliminary cholera culture, in order to produce energetic alkalinization, there is always added to 100 c.c. of nutrient medium, neutralized with phenolphthalein, 2 c.c. of normal sodium hydroxid or 1% crystalline or 0.3% anhydrous soda, in which way many water bacteria are eliminated.

Gruber-Durham test, which is to be looked upon as the most certain reaction which we possess up to this time (see below).

With a negative result in these examinations cholera may still be present, for in very rare cases the occasional absence of vibrios from the stools of undoubted cases of cholera has been proved. Thus, for example, Rumpel failed to demonstrate the vibrios in the first 50 c.c. of rice-water stool from a fresh typical case of cholera.

B. In suspected water.

The water in question is placed in half-filled flasks in quantities of 500 c.c. to 1 liter, together with so much of a strong peptone chlorid of sodium solution (20% peptone, 10% NaCl) that the water contains 1% of peptone; and to this is added also alkali in excess (26 c.c. normal sodium hydroxid, 1% crystalline or 0.3% anhydrous soda). The further examination is carried out exactly as in A, 2–6. Great skepticism is demanded in water examinations.

As especially shown by the detailed work of Dunbar, we may from the first exclude a great number of vibrios resembling cholera in the diagnosis of cholera by means of gelatin plates, potato cultures, photogenesis, etc.; but there were a considerable number of cultures, in which all morphologic and biologic means of separation were lacking, which were pointed out by the **serum reaction**, exactly analogously to the typhoid-coli diagnosis.

This was carried out according to Pfeiffer's method (Z. H. XIX, 75; XX, 198), since at the time of the last active interest in cholera the Gruber-Durham reaction was still undiscovered. Here, unfortunately, all the cultures were excluded which proved to be non-pathogenic for experimental guinea-pigs, and which could not be rendered viulrent by means of the introduction of definite, large doses into animals. (Compare p. 95.)

The cholera serum which is used for these investigations is obtained as follows: A rabbit weighing 1.5 to 2 kilos is injected subcutaneously with the culture substance from three slanted agar cultures (twenty-four hours, 37°), together with about 5 to 6 c.c. of bouillon. The animal becomes somewhat feverish, and on the sixth day is bled, and yields, following the directions on page 105, an active serum, which keeps for months in a dark ice-box if 0.5% phenol is added.[1]

Pfeiffer indicates the working strength of serum as follows: He designates as a titer of serum the smallest quantity of serum which certainly suffices to cause solution of 2 mg. of living normal culture inside of an hour, if it is mixed with 1 c.c. of bouillon and injected into the abdominal cavity of a young guinea-pig weighing 200 gm. The most active guinea-pig serum had 0.5 mg. to the titer. (Serum from four convalescent cholera cases in man had 2.5 to 20 mg. to the titer.)

Of this serum, now, about 10 to 30 mg. (ten times the minimum efficient dose), together with 1 c.c. of bouillon and a loopful of virulent cholera vibrios, are introduced into the peritoneal cavity of a young guinea-pig (200 to 300 gm.). This is accomplished by making a slight cut into the corium with scissors and gently forcing a blunt Koch's syringe through the abdominal muscles. After twenty minutes one removes little drops through the opening with a capillary glass tube.

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[1] If cholera serum is generally introduced as a diagnostic aid, then reliable firms or State institutes must undertake its preparation.

The actively motile vibrios become motionless, swell, dissolve, and in twenty to thirty minutes are dead, or a few may still be alive.

According to the extensive publications of Dunbar (Z. H. XXI, 295), Pfeiffer has the satisfaction of knowing that, by the experiments of himself, Dunbar, Sobernheim, and others, cholera serum has been proved active against eighty-six different true cholera cultures from all parts of the world. With three cultures from cases in man which were considered as cholera by clinicians, R. Pfeiffer obtained negative results, and Dunbar, in subsequent examination, obtained positive ones; he assumed that Pfeiffer had received different cultures. Two other cases could not be reexamined by Dunbar, since the cultures in Hamburg had died.

Negative results were obtained with Pfeiffer's cholera serum in nine cultures from suspected cholera stools (among them three were photogenic), in many vibrios (all photogenic) from water isolated during the prevalence of cholera, and in all varieties found in the Hamburg water since cholera ceased. Dunbar concludes: One may now assert that all varieties which do not react to cholera serum are not cholera vibrios, and it is hoped that we may also some day declare that all varieties reacting to cholera serum are true cholera vibrios.

Gruber and Durham (Münch. med. Wochenschr., 1896, 206, 285) have taught how to make the diagnosis actually more certain in cholera by means of observing the **agglutinating power** of the serum. Serum is prepared as already described, and it is determined in what dilution with bouillon it agglutinates known cholera vibrios. It is usually still active when diluted from 100 to 200 times. (See p. 105.)

Then it is determined whether the organisms which have been isolated and are to be diagnosticated as cholera vibrios are agglutinated by a similar concentration. Gruber and Durham found the reaction rather strongly specific; only a few cultures analogous to the cholera vibrio were agglutinated, and of these, it is at least questionable whether they may not be looked upon as cholera vibrios, as in the case of the Vibrio berolinensis.

Also here the negative result of the test (absence of effect by a serum in dilution of 100, which produced a positive effect against true cholera vibrios when diluted 120 to 150 times) allows an exclusion of cholera vibrios; a positive marked result makes the diagnosis more sure. With a positive but weak reaction the diagnosis of cholera, on the contrary, is not entirely certain; for example, with a tardy action in dilution of 50 times, while true cholera vibrios are promptly agglutinated by dilutions of 80 to 100. It would be best to place no dependence at all upon reactions which only occur in concentration above one in fifty and after half an hour. Compare also Mann (A. H. XXXIV, 179).

Occasionally also the testing of the agglutinating action of the serum upon true cholera vibrios in a case of cholera during the disease or convalescence may make the diagnosis more certain.

Some Other Vibrios Which Are Not to be Confounded with the Vibrio choleræ.

Vibrio spermatozoides. Löffler (C. B. vii, 637).

This remarkable variety, occasionally found in turnip-cabbage infusion by Löffler, and photographed by him, is characterized by powerful terminal flagella (56, VI); the latter disappear or are very delicate upon turnip-cabbage gelatin, but return partially upon reinoculation into turnip-cabbage infusion. The organism presents Y-shaped forkings! (See the note, p. 352.)

Vibrio nasalis. Weibel[1] (C. B. ii, 466; iv, 225).

(Plate 56.)

According to Weibel, a very interesting variety. We have not studied it.

Microscopically: In nasal mucus, thick vibriones (56, II); in bouillon, short, straight rods which stain like the chicken cholera bacteria; upon agar, beautiful screws and bizarre threads (56, III); upon gelatin, almost only the latter are produced (56, IV). They are always without motility. With further cultivation the tenacity of the cultures is rapidly reduced. Upon gelatin plates, when magnified eighty times, there occur minute, yellowish-brown, finely granular colonies with sharp borders. Gelatin stab cultures resemble Strept. pyogenes, the surface growth being minimal. There is no liquefaction. Upon agar the growth is somewhat more luxuriant, and little characteristic; there is a luxuriant growth in nutrient bouillon and bouillon-agar mixture. There is no growth upon potato, and no marked odor. It has no decided pathogenic action. Found in nasal mucus and coating on tongue.

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[1] Also of interest are the following closely related organisms, which have been described by Weibel (*l. c.*) and grow upon gelatin with a yellow color and without liquefaction: **Vibrio flavus** Weibel, **aureus** Weibel, and **flavescens** Weibel. Regarding these varieties, which do not come seriously into question in the differential diagnosis of the Vibrio choleræ, the original must be consulted.

Vibrio lingualis. Weibel (C. B. iv, 227).

According to Weibel, this variety corresponds to the former in absence of motility and liquefaction of gelatin.

Microscopically: Vibriones and threads wavy in one plane, spiral forms do not seem to occur. Gelatin stab culture is somewhat more luxuriant than in the preceding. Upon gelatin plates the deep colonies present a finely threaded border, the threads being coiled and matted, and the colony resembling anthrax to a certain extent. In bouillon there is a flocculent precipitate. It is distinguished from all other known vibrios in that it stains by Gram's method.

2. Spirillum. Ehrenberg, emend. Löffler (C. B. vii, 634).

Long cells, bent into spirals, corkscrew-like, rigid, with usually a unipolar (often bipolar) bunch of flagella.[1]

For a long time only two true spirilla were obtained in pure culture and easily cultivated: Spirillum rubrum v. Esmarch and Sp. concentricum Kitasato. Kutscher (Z. H. XX, 46, and C. B. XVIII, 614) and Bonhoff (H. R. VI, 351) have widened our knowledge of the spirilla species very much, by cultivating from fluid manure and the feces of swine an entire series of spirilla which were already partially known through E. O. Müller, Ehrenberg, and F. Cohn, but were never previously cultivated. Kutscher himself described part of the same with a flat bend to the spiral as vibriones, in spite of the fact that he had stained the terminal, thick bunches of flagella.

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[1] Zettnow (Z. H. XXIV, 72, and C. B. L. IV, 389) has made careful studies regarding the structure of this organism, through which he was led to entirely different results from those of A. Fischer and Migula, which we related on page 20. His results, on the contrary, correspond with those of Bütschli, founded upon many low organisms: ck of a distinct membrane, honeycomb structure of the entire cell, h numerous granules lying within.

The isolation was accomplished by means of agar plates, after a preliminary culture had been prepared which furnished a surface pellicle containing spirilla, according to the method employed in cholera diagnosis. The colonies suspected of being spirilla were torn with a fine platinum wire under the microscope and then it was noticed whether upon slight magnification motion could be observed in the drop of fluid which collected in the rent. If this was the case, it could be conjectured that spirilla (or vibrios) were being dealt with, since the ordinary micro-organisms of manure were almost all non-motile.

Kutscher employed as nutrient medium, meat infusion agar, neutralized with soda, without any further addition. Zettnow finds the additions of 0.1% ammonium sulphate and 0.1% potassium nitrate to be practical, and gives detailed descriptions for the preparation of "spirilla-agar" (C. B. XIX, 393).

Spirillum concentricum.[1] Kitasato (C. B. iii, 72).

(Plate 55, VI–IX.)

Short, more or less winding spirals, 1–8 μ long and 0.5 μ thick, actively motile,[2] staining by Gram's method (55, IX).

Upon gelatin plates delicate, transparent growths, finely punctated (55, VII). In the gelatin and agar stab a spindle-shaped growth below the surface, similar to the Spirillum rubrum, but yellowish. Upon the agar plate, delicate (according to Kitasato, firmly adherent) colonies, opaque and yellowish in the center, and at the border transparent and finely granular (55, VI). Bouillon is rendered moderately turbid. Milk is not coagulated. There is neither formation of gas, nor H_2S, nor indol.

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[1] The name was given by Kitasato on account of the very characteristic cockaded growth in gelatin cultures; our plates show nothing of it.

[2] In spite of every precaution our cultures never showed the active motility observed by Kitasato. We have never tried to stain flagella. Löffler has described terminal bunches of flagella.

On one occasion it was cultivated by Kitasato from putrid blood.

Spirillum rubrum. v. Esmarch (C. B. i, 225).

(Plate 55, I–V a.)

Beautiful threads, more or less elongated or winding, like a corkscrew, often as long as 16 μ; on an average, 1–3.2 μ long and 0.6–0.8 μ thick (55, V). They are motile because of terminal bunches of flagella, and stain by Gram's method. Upon gelatin plates the colonies are at first roundish, almost smooth-bordered, and later they usually have concentric rings with a yellowish-gray central part. The peripheral zones usually appear greenish or reddish. The gelatin and agar stabs grow below the surface in a spindle or cylindric form, being at first grayish-yellow, later rusty brown to red (55, I). In the agar streak there is a very scanty surface growth (55, II). Upon the agar plate the colonies are transparent and slightly crumbly (55, III). Bouillon is rendered faintly cloudy. Gelatin is not liquefied. No formation of gas nor of H_2S. Indol is produced in traces.

On one occasion cultivated by v. Esmarch from a dead mouse. At first it was preferably an anaerobe; after continued culture in bacteriologic collections, it now also grows well at times as an aerobe.

Spirillum rugula. (Cohn.) Lehm. and Neum.[1]

We may add to our remarks upon page 126 in accordance with the investigations of Bonhoff. It is a true spirillum, with thick threads, 8–16 μ long and 1.5–2 μ thick, and is provided with terminal bunches of flagella. Prazmowski's "spores" could not be demonstrated with certainty as such by Bonhoff. Zettnow is convinced that Prazmowski was deceived. Gelatin plate colonies resemble very much those of anthrax; gelatin is never liquefied.

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[1] There appears to be a certain similarity in the cultures to the Vibrio III of Kutscher, which is a thick vibrio provided with bunches of flagella.

Spirillum tenerrimum. Lehm. and Neum.

Spirillum I Kutscher (Z. H. XX, 46). Description according to Kutscher: Short S-forms, very fine and thin, as a rule with three or four turns. Flagella have not been stained. Gelatin plates present characteristic colonies with a compact center; then a finely granular, thinner zone, which carries a row of anastomosing rays at the edge. In the gelatin stab the growth resembles that of mouse septicemia, and also a gradual liquefaction occurs from above. Upon agar plates the colonies are like dewdrops. Slight cloudiness of nutrient media without pellicle formation.

Similar to this is the organism Kowalski has called **Spirillum hachaizæ.**[1] It is a fine spirillum, sometimes seen in the intestine of cholera cases, but also in human dejecta in masses (also often by ourselves in the stools of cases of suspected cholera). Regarding it, there is a large amount of literature, but it is not of much value. Kowalski (C. B. XVI, 321).

Spirillum serpens. (E. O. Müller.) Zettnow (C. B. x, 689).

(Vibrio serpens O. F. Müller, emend. Cohn and Kutscher.)

Quite large spirilla, thin, with usually three or four slight, abrupt turns (the length of two turns is 5–6 μ), and with a terminal bunch of flagella containing as many as fourteen. In the gelatin plate culture are formed macroscopically small starlets which resemble somewhat microscopically those of symptomatic anthrax, but the rays at the periphery are arranged more in a radiating manner, and are only slightly matted. The growth gradually settles down, and in the stab sometimes is accompanied by the formation of an air space. Both upon potato and agar it resembles Bact. coli. The nutrient solution is rendered very turbid, sometimes with a delicate pellicle. Vigorous indol reaction. Our picture (56, I), magnified 1000 times, copied from Zettnow, makes the organism appear very much larger than Cohn's description indicates. Our own descriptions correspond to this.

Spirillum tenue. Ehrenberg, emend. Cohn and Kutscher.

Thin (0.8 μ), markedly winding threads, usually with two to five turns (4–15 μ), with terminal bunches of very delicate flagella. The gelatin plates show the deep colonies as yellowish, round, finely granular, and sharply outlined; the superficial are similar, but more spreading, thin films. The gelatin stab culture presents a delicate growth in the stab, and yellowish, abundant surface growth, with gradual liquefaction and formation of an air space. No growth upon potato. Nutrient fluid rapidly becomes turbid with a thick pellicle. As Kutscher also remarks, Beijerinck's descriptions of three forms of Sp. tenue (C. B. L. I, 1) are not sufficient for identification. Bonhoff found one form deviating somewhat from Kutscher's description; for example, with only two flagella on each side.

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[1] Bonhoff makes the very surprising communication that these fine spirilla are degeneration forms (older forms) of a short organism which grows upon gelatin exactly like the Bact. coli, and, in young cultures, presents the picture of the Bact. coli when magnified 1000 times. The rods have two flagella at one end, do not grow on potato, give the nitroso-indol reaction, do not coagulate milk, and form no gas from grape-sugar (Hyg. Rund. VI, 1896, 351). Further communications regarding this interesting organism are expected, but have not yet appeared.

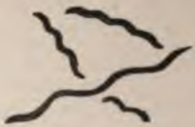

Fig. 19.—Spir. tenue Ehr., after Migula.

Spirillum undula. Ehrenberg, emend. Cohn and Kutscher.

Relatively large threads; usually ½ to 1, rarely 1½ to 3 turns; height and diameter of each turn, 4-5 μ. After longer cultivation there are often scarcely any except straight forms. With terminal bunches of flagella, three to fifteen in number. In gelatin plates there occurs only in the depth a slow growth of sharply outlined, finely granular colonies, beneath which the gelatin sinks a little. In the stab culture development takes place in the upper two-thirds of the stab; the growth on the surface of the gelatin is thin, whitish, slightly lobulated, and after ten days sinks slowly into a depression. Grows on potato. Nutrient fluids uniformly cloudy, without pellicle.

Recently Zettnow and Kutscher have differentiated from this Spir. undula minus also a Spir. undula majus, which is about one-third larger and grows well on meat-infusion gelatin and agar (C. B. XVIII, 614; XIX, 393).

Spirillum volutans. Ehrenberg, emend. Cohn and Kutscher.

Not only the largest spirillum, but one of the largest varieties of bacteria. The threads are about 2–3 μ thick and spirally wound, the height of a turn being 6.6 μ, length 13.2 μ; usually there are 2½ to 3½ turns. In cultures the forms are smaller, similar to the Spir. rubrum. According to Cohn, they have one large flagellum at each end; according to A. Fischer and Kutscher, a terminal tuft of three to eight long flagella, which are often plaited together. The colonies in gelatin plates at first resemble those of Bact. coli; later the gelatin sinks in, and the peripheral parts of the colonies break up. Agar plates are like those of the Bact. coli. In the gelatin stab there is a feeble growth; the surface growth is porcelain white, markedly lobulated, and after ten hours sinks into a depression. Upon potato a dry growth. Nutrient fluid uniformly cloudy, without a pellicle or with a scanty one.

Spirillum stomachi. (Salomon.) L. and N.

Salomon has described (C. B. XIX, 433,) a very interesting beautiful spirillum, which has not been cultivated, and is never absent from dogs' stomachs. It is also found in cats and rats, and can be readily transferred to mice by feeding. It occurs especially in the glands of the stomach.

3. Spirochæte. Ehrenberg.

The cells are flexible, and present long, pointed, spirally bent threads. Flagella are unknown. Motility is assigned to an undulating membrane.

A key for their differentiation may be omitted, since only two or three species are known.

Spirochæte Obermeieri. F. Cohn.[1]

(Plate 56, VIII and IX.)

Literature.—Obermeier (C. f. med. Wiss., 1873, 145); Koch (Mitt. a. d. Ges.-Amte, I, 167); Soudakewitsch (A. P. V, 545); Cohn (Beiträge, I, Heft III, 196); literature by Afanassiew (C. B. XXV, 415). The personal investigations of these authors are not adapted for use in a text-book.

Bacteriologically very little is known. Large, flexible, motile threads, coiled like a corkscrew, with pointed ends, $1\frac{1}{2}$ to 26 times as long as the diameter of a blood-cell, usually 20–30 μ. Flagella and spores are not known.

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[1] Sakharoff discovered, in the blood of geese suffering from an epizootic disease in Caucasus, a motile but not flexible spirochæte,—**Spirochæte anserina** Sakharoff (C. B. XI, 203),—through which the disease may be transferred to healthy animals. Details are given regarding it by Gabritschewsky (C. B. XXIII, 365). It was not cultivated. The following may be simply mentioned: **Spirochæte plicatilis** Ehrenberg from marsh-water and the **Spirochæte of the saliva,** which have been often seen but never cultivated. According to F. Cohn (Beiträge, Bd. I, Heft II, and Heft III, pp. 197, 199), these varieties are not to be distinguished microscopically from the Spiroch. Obermeieri.

It is typically found in the blood and spleen of recurrent fever cases, hardly ever during the afebrile periods (an exception proved by Naunyn); demonstrated by Karlinski to be the cause of a part of the cases of febrile icterus (C. B. XI, 26).

It stains readily with the usual anilin dyes. Günther recommends that the dried and fixed preparation be previously freed of part of the albuminous bodies by means of a 1% to 5% acetic acid solution. It is not stained by Gram's method.

No cultures have so far been successful. According to Pasternatzky, the spirochæte may be preserved alive for about ten days if a leech is allowed to fill itself from a case of recurrent fever, and then is kept upon ice.

Inoculation experiments have succeeded only upon man and monkeys. The monkeys become sick after about three and a half days, but present only the initial attack of fever and no recurrence. Extirpation of the spleen makes the disease more dangerous for the monkey.

APPENDIX I.

Actinomycetes.

For the limitation of this group and its genera, see page 127.

We have conscientiously recorded all that is known to us in the literature regarding forking, branching, etc., in other forms up to this time considered as true bacteria; thus, B. pyocyaneum, B. influenzæ, B. tetani, B. radicicola, Vibrios,—the cladothrix form of B. murisepticum was immediately retracted by Kitt himself,—and we must naturally acknowledge that these observations make it more difficult to perceive in the branching a distinguishing peculiarity between actinomycetes and fission-fungi. Innumerable similar difficulties are, however, encountered in the definition of higher plant families—some genera are often placed with equal propriety in one or another family. If at a later time, because of further investigations, the significance of branching should be construed in a manner different from that of to-day, it will still remain true in any case that the actinomycetes of to-day, which we have collected in part upon the basis of branching, will form a perfectly natural family, even if their family diagnosis should be essentially remodeled.

1. Corynebacterium. Lehm. and Neum.

Cultures having throughout the character of cultures of true bacteria; soft, lying flat and loose upon the nutrient media. Stain well with the ordinary bacterial stains, but are not acid proof. *Microscopically:* Rods, which frequently present clubbed swellings at the ends, appear more or less distinctly composed of differently staining segments, and in many cultures present constantly an undoubted, true branching.

Key to the Recognition of the Most Important Varieties of the Genus Corynebacterium.

1. Plate cultures upon gelatin: Colonies like those of Bact. coli or typhi—*i. e.*, roundish, and when magnified sixty times, with distinct lineal markings; upon agar and serum-agar, just like Bact. coli. Potato culture brownish-red. Cause of glanders. **Corynebact. mallei** L. and N., page 384.

2. Plate cultures upon agar and serum-agar with very characteristic granulation (splintery!). Growth upon potato usually slight. Colorless to yellowish.

(*a*) Very luxuriant growth upon the nutrient media, even upon potato. Gelatin gradually discolored brown. Growth often yellowish, sometimes brownish. Not pathogenic for animals. Usually little acid produced in bouillon. Usually no granules in the rods when stained by Neisser's method. **Corynebact. pseudodiphtheriticum** (Hofmann-Wellenhof) L. and N., page 404.

(*b*) Growth of medium intensity upon agar, and best upon serum-agar; poor upon gelatin and potato. Vigorous production of acid in bouillon. Usually granules in the rods when stained by Neisser's method. Pathogenic for man and animals. **Corynebact. diphtheriæ** Löffler, L. and N., page 389.

(*c*) Scanty growth upon nutrient media. No production of acid in bouillon. No granules staining by Neisser's method. Not pathogenic. **Corynebact. xerosis** (Neisser and Kuschbert) L. and N., page 406.

The close relationship of Corynebacterium diphtheriæ with Coryn. pseudodiphtheriticum and Coryn. xerosis permits no certain recognition from this key alone. (Compare p. 403.)

Corynebacterium mallei. (Löffler and Schütz.) L. and N.

(Plate 57.)

Common Names.—Glanders bacillus; German, Rotz; Latin, malleus; French, morve; English, glanders. Bacillus mallei Flügge.

Principal Literature.—Löffler (A. G. A. I, 141). Kranzfeld (C. B. II, 273). Kitt (C. B. II, 241).

Microscopic Appearance.—Slender rods (2–3 μ long, 0.4 μ thick), sometimes with brightly shining bodies (metachromatic bodies), which may be shown very well by means of Neisser's staining method for diphtheria granules. True endogenous spores are never present; all previous positive statements to the contrary are erroneous. In old cultures clubbed, vesicular enlargements which create the impression of involution forms are often seen; also long threads, which sometimes exhibit true branching (57, XII) in great abundance. See Semmer (C. B. XVIII, 68). Dissertation by Erich Wolf, Würzburg, 1898, and Marx (C. B. XXV, 274).

Staining Properties.—Somewhat difficultly with ordinary stains; does not stain by Gram's method. For staining the bacteria in sections, Nicolle's method is to be recommended (Technical Appendix).

Requirements as Regards Composition of Nutrient Media, Supply of Oxygen, and Temperature.—Grows best at incubator temperature (minimum, 25°; maximum, 40°). Prefers glycerin-agar to ordinary agar, but is not particular. Grows well aerobically, poorly or not at all anaerobically.

Gelatin Plate.—(*a*) *Natural size. Superficial and deep colonies:* Small, whitish, punctiform; also after a long time they do not become essentially larger. The superficial ones have a delicate, transparent halo (57, V).

(*b*) *Magnified sixty times. Superficial:* Irregularly roundish; scalloped, wavy margin; shining white, transparent, with wavy elevations and marked reflex. Old colonies are more yellowish, especially in the center, with linear, depressed markings. They are very similar to colonies of B. typhi and putidum in the early stages (57, VIII, e). *Deep:* Roundish or oval; sharply outlined; in the center delicately crumbly, at the outer part streaked. The peripheral zone is sharply marked (57, VIII).

Gelatin Stab.—*Stab:* Thread-like; sometimes faintly granular, sometimes like a string of pearls; gray. *Surface growth:* Exceedingly delicate, perfectly transparent, gray, with a ragged fringe, and of a dull luster (57, I).

Agar.—Not distinguishable from Bact. coli; entirely non-characteristic (57, IV, VII). For a year we cultivated a form of the Cory. mallei, which occurred spontaneously and which produces rusty-brown colonies upon agar. This is a counterpart to the rusty-colored streptococci mentioned on page 137.

Bouillon Culture. — Almost clear, moderate homogeneous sediment, which rises up uniformly upon shaking.

Milk.—Slowly coagulated.

Potato.—At first there is a light yellow or brownish growth, with a moist luster, scarcely at all or slightly elevated, lighter at the edge, not sharply outlined (57, x). After a longer time: brownish-yellow or brownish-red, smooth, wavy border, sharp outline, the periphery still being paler. The potato becomes discolored (57, IX). The culture has much similarity to that of the Vibrio choleræ. Cultures upon carrots present a white growth and were employed by Marx (being protected from drying) for his studies upon branching.

Resistance to Drying.—Slight. At 25° dead in ten days (Bonome). According to Bonome, it withstands 70° for six hours without injury (!) ; 70°–75° kills in five or six minutes ; 90°–100°, in three minutes.

Chemical Activities.—Except for the formation of pigment upon the potato and a trace of indol in bouillon, nothing is known beyond the formation of mallein (bacterioprotein). Forms no H_2S. No gas is formed from carbohydrates.

Distribution.—

(*a*) *Outside the body:* Never has been found.

(*b*) *In healthy body:* Never has been found.

(*c*) *In diseased human organism:* Man is fairly susceptible to glanders, almost always the transfer occurring from horses. About 50% of the cases die. The bacteria are found in the secretion from the ulcers of glanders and in the glanders nodules. The principal places of infection are the skin and mucous membranes. The glanders bacilli also enter the uninjured skin through the hair follicles and spread in the lymph-spaces. Chronic glanders also occurs in man, although very rarely.

(*d*) *In animals:* Of our domestic animals the following are attacked : Horse, ass, cat (and the wild canines of the zoological garden); according to infection experiments, also dogs (especially in the young), goats, sheep, rarely swine are susceptible. Cattle and birds are immune. According to Schütz, there is no primary pulmonary glanders; on the contrary, the lungs are always affected secondarily from the skin or mucous membrane. The primary port of entry in the skin or nasal mucous membrane is often already healed when the pulmonary glanders begins. According to Nocard, the transparent gray nodules in the lung, which show a tendency to calcify, are due to glanders infection. Schütz has (always?) found a small roundworm in them, and denies that they are connected with glanders (C. B. XXIII, 901).

Experimental Observations Regarding Pathogenic Effects.[1]—(*a*) *On animals:* For experimental purposes the guinea-pig is best, and next the field mouse (Arvicola arvalis) (Löffler). Also the following may be used (Kitt): Mus sylvaticus (wood mouse) and Arvicola amphibius (water rat). The rabbit is slightly susceptible. Immunity exists in the case of gray and white house mice[2] (Löffler) and rats. Experiments upon cats and dogs have more disadvantages than advantages.

The most important animal experiment is the injection of 2 c.c. (not too little) of a suspension of the pure culture or of the crushed, suspected organ through the median line above the bladder into the abdominal cavity of a male guinea-pig (Straus, Arch. de Med. exp., I, 1889, 460). After forty-eight to seventy-two hours there is presented a marked swelling, redness, and tenderness of the scrotum as a pathognomonic symptom of the successful transfer of glanders. The swelling is dependent upon the formation of numerous glanders nodules upon the tunica vaginalis testis, the two layers of which are stuck together by a purulent exudate; and glanders nodules also occur inside the testicle. After twelve to fifteen days, sometimes even four to eight days, the animals die, before which suppuration in the testicle may have discharged externally. To expedite the diagnosis, the diseased testicle may be examined even before the death of the animal by means of potato cultures, etc. Subcutaneous injections are not to be recommended in guinea-pigs, as the abscesses which form primarily imperil the experimenter by opening externally, and death occurs only after twenty-five to thirty days (also here almost always the testicles are diseased).

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[1] Experiments are permissible only in well-equipped laboratories and with most extreme precautions

[2] According to Shattock, they become sick only at a later time, and die after two or three weeks (C. B. XXV, 323).

(*b*) *Upon man:* No purposeful experiments have been made with glanders bacilli in man. A number of fatal laboratory cases indicate the great danger for man of the pure culture.

Special Methods for Demonstration and Cultivation.—Acute cases of glanders in horses are usually not difficult to diagnose from the clinical symptoms. The diagnosis in subacute and chronic cases is harder and often very difficult, even after autopsy and with the additional help of bacteriologic aids.

(*A*) *In the case of living animals the following is recommended:*

1. Mallein—the protein of the glanders bacillus—is injected subcutaneously. While healthy animals remain afebrile or show only a slight fever of reaction, those affected with glanders usually show a gradual elevation of the temperature of 1.5°–2°;[1] and after it has remained at the highest point for a short time, it gradually falls. At the point of the injection there remains a swelling for several days if the animal is affected with glanders. The method furnishes no absolutely certain diagnostic proof, since sometimes the febrile reaction occurs in healthy individuals, or remains slight in diseased ones. Most authors recommend it highly.[2]

2. The suspected nasal cavity is wiped out with a cotton swab and 1 c.c. of a suspension of the material thus obtained is injected intraperitoneally into a guinea-pig (see p. 387).

3. One of the swollen, paratracheal lymph-glands is

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[1] The elevation of temperature is the more significant, the higher the original temperature. An elevation of more than 2° with a high initial temperature is fairly certain proof. An elevation of temperature not over 1.1° indicates an absence of glanders; 1.2°–1.9° is suspicious. See Eber (C. B. XI, 20).

[2] The experiences of Prof. Schütz make one especially skeptical. This is particularly true in his latest results with 64 horses: 9 out of 61 healthy horses reacted, while the 3 with glanders did not!

extirpated and smear cultures prepared from the same (incubator):

(*a*) Upon potato (brown color of the growth).

(*b*) Upon glycerin-agar.

Also a microscopic preparation is made, and, further, a guinea-pig is injected.

(*B*) *In the case of living men:* The secretions from glanders ulcers are best examined by infections of guinea-pigs.

(*C*) *In animals at the autopsy:*

1. Cultures and animal investigation with fresh, crushed glanders nodules.
2. Staining of sections of glanders nodules (difficult).

Kutscher (Z. H. XXI, 156) has described an interesting **pseudo-glanders bacillus.** It grows similarly to cholera upon gelatin, luxuriantly upon agar, white and dry upon potato. Microscopically it resembles the B. mallei absolutely, but stains by Gram's method. It is interesting that, if injected intraperitoneally according to the method of Straus, it produces a swelling of the testicle in male guinea-pigs, as the B. mallei does. The swelling is due more to nodular swelling of the coverings of the testicle than to swelling of its substance. The animals usually die after four or five days, when a peritonitis (often hemorrhagic) dominates the picture. There are no nodules in the other abdominal organs, but the omentum is always rolled up and highly inflamed.

Corynebacterium diphtheriæ. (Löffler.) L. and N.[1]

(Plates 58, 59, and 60.)

Synonym.—Bacillus diphtheriæ Löffler.

Common Names.—Diphtheria bacillus, Löffler's bacillus. "Löffler."

Literature.—Löffler, Mitt. a. d. Ges. Amt., Bd. II. Complete list of literature up to 1894 is found in the thorough work of Escherich: Aetiologie und Pathogenese der epidemischen Diphtherie, Wien, 1894. Latest literature: Heinersdorff, Arch. f. Ophth., Bd. 46, p. 1. Especially important also are: Neisser (Z. H. XXIV, 443) and Kurth (Z. H. XXVIII, 409). C. Fränkel(Berl. klin. Wochenschr., 1897, 1085). Zupnik, *l. c.*

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[1] The statement of Zupnik (Berl. klin. Wochenschr., 1897, No. 50), that the diphtheria bacillus may be separated into two varieties, could not be verified by Slawyk and Manicantide (Z. H. XXIX, 181). Zupnik divides them as follows:

(*a*) Relatively large, flat, dull agar colonies, of irregular contour. They stain by Gram's method, are non-motile, and are fully virulent for guinea-pigs. Bouillon is not cloudy and only presents the formation of a pellicle.

(*b*) Smaller agar colonies; circular, conically elevated, shining. They do not stain by Gram's method, are sluggishly motile (!), and in guinea-pigs produce only infiltration and necrosis, and never death.

Microscopic Appearance.—Slender, rather long, rods, often a little bent and usually somewhat swollen at one or both ends. Many times they are arranged in pairs. With Escherich, the following forms may be distinguished:

1. Wedge-shaped rods, about 1.5–2 μ long, about 0.5 μ thick (60, II, IV).
2. Long cylindric rods (especially upon agar and potato) (60, I), 3–4 μ long, 0.4–0.5 μ thick.
3. Rods with clubbed swellings (especially upon serum), as much as 6–8 μ long. The clubs reach a diameter of 1.0 μ (60, III).

In 1 and 3 the thin ends are often long and drawn out to a point. The same culture upon alkaline bouillon forms long clubbed rods; upon acid bouillon forms short, wedge-shaped rods. The short forms are more often parallel in arrangement; the long, more at angles, and arranged in rosettes like fingers, etc.

According to Kurth, the probability of the form under observation being pathogenic is increased if it can be established that in contact preparations, from young cultures (six hours at 35°) on Löffler's serum, there are present at least a number of longer forms (seven times as long as thick) or V-shaped forms. Further, Kurth attaches value to an appearance of the young rods being so arranged as to suggest the fingers of two hands, spread out upon each other.

They have recently been often observed to grow into unbranched threads (in part with clubbed swelling at the ends), and even into branched threads (Babès, Klein, C. Fränkel, C. B. XVII, 896). We have also possessed cultures which presented striking branching forms in pre-

Bouillon is first rendered diffusely cloudy, then becomes clear beneath the pellicle.

Slawyk and Manicantide found thirty completely investigated pathogenic cultures to correspond to the plan, only many of them presented more of the smaller, glistening, elevated agar colonies ponderance (60, XII). The other forms represented in Plate 60, V–IX, also occur in true diphtheria, the short forms especially in very young cultures.

Schütz found the best formation of branches in different cultures to occur sometimes upon albumin, sometimes on glycerin-agar; also, in distinction to C. Fränkel, he often observed beautiful branching in bouillon (Berl. klin. Wochenschr., 1898, 297).

Motility.—Never present. We have never seen any motion at all.

Staining Properties.—Stains with all the anilin dyes, especially in young cultures; also by Gram's method. Gram's method, especially as modified by Czaplewski, is to be recommended for the study of smear preparations from diphtheria material; carbol gentian-violet and Gram's solution of iodin are used. (See Technical Appendix.)

Carbol-fuchsin and anilin gentian-violet solution stain very intensely, without revealing the finer structure. Staining with warm Löffler's methylene-blue solution and differentiating in water reveals a very characteristic structure in the bacilli. They consist of alternating sections of intensely and faintly stained substance surrounded by a delicate envelope of faintly stained material. This is most marked in older cultures on blood-serum. Very young bacteria stain uniformly blue.

Metachromatic Granules.—Max Neisser has pointed out that the occurrence of metachromatic granules permits the differentiation of the diphtheria bacillus from many related forms. According to Neisser, cultures are employed which are made upon Löffler's serum and kept at 35° (not warmer) for nine to twenty hours (in older cultures the granules disappear in part). The dried preparation is stained for one to three seconds (Auckenthaler found ten to fifteen seconds to be often better) with acetic acid methylene-blue solution (Technical Appendix), washed in water (tap-water should only be used if it does not contain much free CO_2), and then counter-stained three to five seconds with a weak solution of Bismarck brown (Technical Appendix). There is then observed a blue granule at one or often at both ends in a majority of the brown-stained bacilli; not infrequently there are more than two such granules (60, x, xi). Nevertheless virulent diphtheria bacilli without granules occur, although very rarely (see Kurth, *l. c.*), so that a lack of the granules does not exclude the diagnosis of diphtheria. (See also p. 402.)

Relation to Oxygen.—Optimum growth with entrance of air; when oxygen is excluded, the growth is lessened.

Requirements as Regards Temperature, Reaction, and Composition of the Nutrient Medium.—It grows well and abundantly at incubator temperature only. Optimum temperature 33° to 37°; extremes, about 18°–20° and 40°. Glycerin-agar is more favorable for its growth than ordinary agar, but serum or ascitic nutrient medium are much better. Löffler's blood-serum mixture is much used (Technical Appendix); also Tochtermann's and Deyke's nutrient media are highly recommended (Technical Appendix). Since we have used glycerin-ascites-agar almost exclusively instead of glycerin-agar, we have obtained excellent results, but one must become accustomed to the relatively luxuriant appearance of the growth. Upon gelatin at 22°–24° the growth is so absolutely without characteristics (no liquefaction), and so scanty, that such cultures are never prepared.

Gelatin Stab.—Along the stab canal only a slight growth. The surface growth is yellowish-white, a little elevated, with a smooth wavy border and in part lobulated. It is faintly shining.

Glycerin-agar Plates.—(*a*) *Natural size:* Circular or roundish colonies, white to dirty yellow. The border is smooth, they are more or less elevated, and with a moist or faint luster. Many cultures present more luxuriant (58, vii *a*) and many more delicate growths (58, vii *b*).

(*b*) *Magnified sixty times:* The colonies present their characteristic form after twenty-four hours at 37°. They are small, roundish, usually exceedingly transparent colonies of a grayish-yellow or brownish color. At the periphery they are usually split or torn, and almost without exception are markedly crumbly. Many colonies appear at the periphery as if raveled out. Still, according to the culture, they are thinner or thicker, lighter or darker, coarsely or finely granular (59, i *a* and *b*). After two days the colonies are thicker, somewhat irregular at the periphery, and, when magnified a little more highly, distinct single rods are seen to project at the edge. The center is opaque yellowish-brown (59, II *a* and *b*). In still older cultures dark irregular spots occur, the colonies become yet more crumbly, the periphery more torn, and the inner part more opaque (59, III). Colonies occur, however, especially upon better nutrient media (ascites-glycerin-agar), which are rounder, thicker, and therefore more opaque from the first, and finely granular (59, VI *a* and *b*). After a longer time such luxuriant colonies resemble perfectly those of cocci or sarcinæ (59, VII). Also all the other forms reproduced in Plate 59 may occur as they are found in closely related non-pathogenic forms.

Glycerin-agar Streak.—The same may be said of it as of the growth upon glycerin-agar plates. There occur also here more luxuriant and more delicate forms (58, I and II). Especially upon glycerin-ascites-agar the cultures are sometimes so luxuriant that they resemble those of the Bact. coli or micrococci. (Compare 58, III and IV.) In many cases after two to six weeks the agar shows a brown discoloration.

Blood-agar Streak.—Very good growth.

In raw hens' eggs there is abundant growth, and upon cooked white of egg there is a relatively luxuriant growth.

Serum Culture.—Löffler's coagulated blood-serum mixture is often employed for cultures. It consists of the serum of calves or sheep (or slightly alkalinized serum of cows) to which is added one-third its volume[1] of neutral veal bouillon (containing 1% peptone, 1% grape-sugar, and 0.5% sodium chlorid). We find this nutrient medium to possess about the same advantages as glycerin-ascites-agar.

Bouillon.—After twenty hours a cloud is deposited, either in the form of fine, dust-like granules upon the sides and bottom of the tube, or (and what most authors give as most frequent, but Escherich only seldom found in Gratz) fine flocculi form, which are easily precipitated, and, upon shaking, rise again. Both types are connected by transition forms. Young cultures usually present delicate, old ones thick pellicles.

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[1] Escherich recommends one-fourth or one-fifth in order to insure certain solidification of the serum upon heating.

Alkaline bouillon first becomes acid, then alkaline again, the latter change being favored by passing air through it. (See Chemical Activities.) The diphtheria bacilli grow poorly upon bouillon which has been long stored, and in such a case the nutrient value is increased by boiling it (Escherich).

Milk.—Luxuriant growth usually occurs without coagulation. They live a long time. Reaction amphoteric. According to Schottelins, this is especially true of raw milk, cooked milk being much less favorable (C. B. XX, 897).

Potato.—Upon acid potato very poorly or not at all; upon alkaline potato after eight to fourteen days, a very scanty growth. It appears only as a delicate, shining, sharply limited veil, which sometimes may be lifted with a platinum needle. A more luxuriant growth of the diphtheria bacillus upon potato occurs, although only rarely (58, IX).

Special Nutrient Media.—In non-albuminous urine (Guinochet) which has been sterilized and rendered faintly alkaline the diphtheria bacillus grows slowly, but it is pathogenic. Schloffer (C. B. XIV, 657) recommends urine-agar (a meat infusion-peptone agar—2%—is mixed with fresh, sterile urine). According to Gamaleia, a good nutrient medium contains glycerin, 40 parts; meat extract, 5 parts; sodium chlorid, 5 parts; and water, 1000.

Spore-formation does not occur.

Viability.—(*a*) *In the body:* It is found in the throat for weeks or even for two months after convalescence from diphtheria in many cases (Löffler, Abel).

(*b*) *In cultures:* If kept cool and in the dark, for from six months to one and one-half years. In the incubator they usually die after one to three months because of drying. In well-closed bouillon cultures they remain alive also in the incubator for one year or longer.

(*c*) *In water and foods:* See Montefusco (C. B. XXI, 352).

Resistance to: (*a*) *Drying:* Very considerable. Pure cultures on silk threads in the room remain alive three or four weeks, and under favorable conditions for months. In dried diphtheria membranes they live as long as three months. Even when dried so that it may be pulverized into dust, the bacteria remain alive and infectious (Germano).

(*b*) *Moist heat:* They are rapidly killed at 60°, and in a few hours at 50°.

(*c*) *Cold:* When dried, many individuals bear the cold of the German winter for two and one-half months without reduction of virulence (Abel); according to Kasansky, cultures endure the Russian winter for months.

(*d*) *Light:* While germs suspended in water are destroyed in a few hours (two to eight hours) by direct sunlight, agar and especially bouillon cultures stand the sunlight for six hours very well.

Chemical Activities.—(*a*) *Formation of gas and acids from carbohydrates:* From grape-sugar, even from the minute quantity found in ordinary bouillon, easily demonstrable acid is produced; also similarly from glycerin.

The increase of acid produced by typical diphtheria cultures in 5 c.c. of non-saccharine bouillon after twenty hours usually amounts to 1.2–1.5 c.c. of 1 : 40 normal sodium hydroxid; after forty hours, 2.5–3.0 c.c., phenolphthalein being used as indicator. In 1% sugar bouillon we found about twice as much acid formed : *i. e.*, 2.6–3.8 in twenty hours and about 6.0 in forty hours. Kurth, like Spronck, proposes that 0.2% grape-sugar be always added to bouillon, since he often obtained bouillon which contained too little sugar.

(*b* and *c*) Production of H_2S is slight. Indol is always produced.

(*d*) In older cultures there is some nitrite, so that the "cholera-red reaction" is obtained with sulphuric acid alone (Palmirski and Orlowski).

(*e*) *Chromogenesis:* Rarely there occur yellow to red cultures. (Zupnik, Fränkel. See p. 405.)

(*f*) *Toxins:* Old bouillon cultures filtered through clay produce symptoms identical with those following inoculation of the diphtheria bacillus itself [1] (Roux and Yersin).

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[1] The fibrinous exudate alone is lacking at the place of inoculation. Often there occurs albuminuria, diarrhea, and very irregular action of the heart. During the course or after the disappearance of the acute symptoms paralyses occur, especially in the more resistant animals:

According to Dungeren, especially active toxins are obtained by the addition of ascitic fluid to bouillon (C. B. XIX, 137). The addition of sugar to bouillon is to be avoided (Sprouck, A. P., 1895, 758). So long as bouillon cultures are of acid reaction they contain no toxin ; usually the toxic action corresponds to the increase in alkalinity (Hilbert, Z. H. XXIX, 157), but not always (Madsen, Z. H. XXVI, 157). According to Roux and Martin, the formation of toxin is favored by the entrance of oxygen (large surface of bouillon). Regarding this point, see also Hellström (C. B. XXV, 217).

The toxins are precipitated by alcohol, and are scarcely at all dialyzable. Precipitates of calcium phosphate (from the addition of calcium chlorid to bouillon) carry them down also. Temperatures above 60° rapidly reduce the toxicity. With alcohol and vacuum apparatus the toxins may be obtained as a powder. Toxins are produced not only upon albuminous, but also upon non-albuminous nutrient media, alkaline urine (Guinochet), and Uschinsky's nutrient medium (p. 75). According to H. Kossel, the diphtheria toxin is formed in the bodies of the microorganisms and at once secreted (C. B. XIX, 977).

The bodies of the bacteria contain no large quantity of toxin. Regarding the chemistry of the toxins, see page 73; also, regarding their resistance and other properties, see Fermi (C. B. XV, 303). For the most recently advanced division of the diphtheria toxins by Ehrlich—prototoxoid, syntoxoid, epitoxoid—the original article must be consulted (Deut. med. Wochenschr., 1898, 597). In distinction to tetanus, the emulsion of the brain and spinal cord of susceptible animals has no antitoxic action against diphtheria toxin (Bomstein, Aronson).

Distribution.—(*a*) *Outside the body:* Upon things used by the diphtheria patient (linen, brushes, playthings, walls and floor of room). On the hair of nurses. The air never

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rabbits, pigeons, dogs, cats, rarely guinea-pigs. Most characteristic are the paralyses which first appear after apparent recovery of the animal from the acute symptoms of intoxication (post-diphtheritic paralyses). The susceptibility of animals to the diphtheria poison is much increased by hunger, exhaustion, etc. (Valagussa and Ranelletti, C. B. XXIV, 752).

contains living diphtheria bacilli (except from a momentary contamination by the coughing of the patient—Flügge).

(*b*) *In healthy body:* Sometimes found in the mouth and nasal cavities, also in the conjunctival sac of healthy persons, especially in those coming in contact with diphtheria cases. In a diphtheria epidemic in a barracks, Aaser found diphtheria bacilli in the throats of 19% of the occupants who were healthy.

(*c*) *In diseased human organism:* Are found without exception on the outer side (the side toward the cavity of the mouth) of the diphtheritic membranes[1] of recently affected men, and with more difficulty and less regularly in chronic cases.

Principal localizations: Throat, nose, larynx, trachea; more rarely, stomach, defects in skin and muscle (wounds), and vagina.

The wide-spread assumption that the diphtheria bacilli are to be found only at the local seat of disease is unfounded. Lately they have been found rather frequently (also in man) in the blood and internal organs, especially the spleen and kidney (Frosch, Z. H. XIII, 49; Nowak, C. B. XIX, 982). Recently also rhinitis fibrinosa, conjunctivitis crouposa (severe and very mild forms), and many middle-ear suppurations have been traced to the diphtheria bacillus.

Almost regularly the Streptococcus pyogenes accompanies the diphtheria bacillus (Löffler) and in the pathologic process plays a synergistic rôle.

Regarding the significance of mixed infection, Bernheim has ascertained:

1. The metabolic products of the streptococci favor the growth of diphtheria bacilli and increase the virulence; also the production of toxin by the diphtheria bacillus is increased (Hilbert, Z. H. XXIX, 157).

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[1] Diphtheritic angina also occurs without formation of membrane. On the other hand, not rarely clinical "diphtheria cases," in spite of a perfectly typical local symptom-complex, present no diphtheria bacilli (according to Escherich, in Gratz about 25%). A number of other organisms (for example, streptococci) can cause the symptoms of diphtheria of mucous membranes. The mortality in these cases is minimal. Also "wound diphtheria" may depend upon streptococci or Bact. coli.

2. Mixed infection with streptococci and diphtheria bacilli is more dangerous for the animal than pure diphtheria infection.

Nevertheless the diphtheria bacillus alone may undoubtedly produce all the clinical symptoms of sepsis (Genersich).

(*d*) *In animals:* Certain spontaneous disease produced by Löffler's bacillus has never been observed in any animal. The susceptible guinea-pig is immune to the diphtheria bacillus introduced by feeding, by inhalation, or by swabbing. Spontaneous disease (diphtheric bronchopneumonia) is said to occur in cats (E. Klein, C. B. VIII, 7). Klein claims also to have observed spontaneous diphtheria in milk cows, in which, moreover, the diphtheria bacilli escaped in the milk.

The spontaneous diphtherias of hens, pigeons,[1] and calves always (?) have other causes. (Compare Löffler, Mitt. G. A. II; Ritter, H. R., 1896, 839).

Still, certain of the causes of "animal diphtheria" appear to be transferred to man. Consult the well-known observation of Gerhard (II. Kong. f. innere Med.), and also Galli-Valerio (C. B. XXII, 500: extensive critical review of literature).

Experimental Observations Regarding Pathogenic Effects.—(*a*) *Upon animals:* The virulence of freshly isolated cultures varies greatly; in general, severe cases furnish virulent cultures and mild ones cultures with slight virulence; still, there are exceptions. Experimental and accidental (cultural) attenuation is often observed. Roux and Yersin assert that there occurs a regular, striking reduction of virulence in the last few diphtheria bacilli demonstrable during convalescence. It was not found so by Escherich, and still other writers cultivated virulent bacilli from convalescents long after the clinical symptoms disappeared. A good standard for the virulence of a culture[1] consists in the toxicity of the filtrate of a culture of a certain age.

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[1] Gallez claims to have positively demonstrated in Belgium that, besides the "fowl diphtheria," which has nothing to do with human diphtheria, there is also a "fowl glanders," which is caused by attenuated Löffler's bacilli (H. R., 1896, VI, 472).

In the interest of rapid work, Escherich recommends for the estimation of virulence a statement of the quantity, expressed in percentage of the body-weight, of feebly alkaline twenty-four hours' bouillon culture which just suffices, when introduced subcutaneously, to kill a guinea-pig with acute diphtheria. With 1.5 c.c = 0.5% of the body-weight, Escherich never obtained a negative result; with his most virulent cultures, 0.1 to 0.3 c.c.—*i. e.*, about 0.05%—sufficed. Aronsohn has cultivated still more virulent bacteria, of which 0.02% to 0.025% of bouillon filtrate was certainly fatal.

Also, for infection experiments[2] the best animal for use is the guinea-pig. Death is caused by 0.02 c.c. of a virulent culture in two days; by 0.01 c.c. in three or four days. Usually 0.5 to 1 c.c. is injected. About twenty-four hours after the subcutaneous injection the following picture develops: The animal is weak, without appetite, the hair bristling, snout cold and bluish, respiration very harsh. There is infiltration at the place of injection, and often also for some distance beyond. Death occurs after twenty-four to sixty hours. There may be entire absence of special symptoms of disease except loss of weight.

Autopsy: At the point of injection a whitish infiltration surrounded by hemorrhagic edema, and, in chronic cases, callosities discolored by hemorrhage. The most important changes in the internal organs are: Suprarenal capsules hyperemic; exudate into pleuræ, often also into pericardium; spleen unchanged; often parenchymatous nephritis and myocarditis. The upper part of the intestine is reddened. Escherich observed cultures with which the inoculation was never followed by pleural exudate. In these experiments an increase of the bacteria occurs almost exclusively locally, and only rarely can they be cultivated from the internal organs.

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[1] See De Martini (C. B. XXIV, 420) regarding occasional discrepancies of toxin formation and infectiousness in the same culture.

[2] In order to recognize diphtheria bacilli of doubtful and very slight virulence as still virulent, Trumpp injects them simultaneously with a sublethal dose of diphtheria toxin. The animal must die, in contrast to a control animal, and with reinoculation of definite quantities into new animals the virulence must constantly increase, so that finally the inoculated animals die without any additional diphtheria toxin (C. B. XX, 721).

Subchronic and chronic cases (death sometimes occurring only after months) present changes in the internal organs which are less marked, or no alterations at all are found. At the point of injection all changes may be lacking or ulcers may follow necrosis of the skin. The animals are always emaciated and very much reduced in weight. Escherich never saw postdiphtheritic paralysis in experimental animals; other authors have occasionally.

Rabbits are much more resistant to subcutaneous inoculation than guinea-pigs; white mice and rats are almost immune. On the contrary, cats, dogs, and cows are susceptible. Of birds, young pigeons and small birds (finch, siskin, etc.) are especially susceptible; hens less, and only when young.

Diphtheritic diseases of mucous membranes analogous to those observed in human diphtheria may be produced by rubbing diphtheria bacilli into the slightly injured (not the uninjured) mucous membrane of the trachea and conjunctiva of rabbits, of the throat of monkeys, of the throat and larynx of pigeons and hens. The disease process and membrane formation remains local. The best results follow inoculations upon the vaginal mucous membrane of guinea-pigs (Löffler): If one pulls apart the vagina, which is always feebly adherent, and places a pinhead-sized quantity of diphtheria bacilli upon the mucous membrane, which has always received a minimal injury in the manipulation of separation, on the following day there is marked redness and hyperemia, and after forty-eight hours the formation of a thin, closely adherent covering can be demonstrated. This infection may terminate in recovery or death.

Roger and Bayeux produced, by the injection of $\frac{1}{4}$ to 1 drop of diphtheria poison into the trachea of rabbits, beautiful diphtheritic membranes, while guinea-pigs die too soon for it to appear.

(*b*) In man there have been no experiments.

Immunization.—Animals may be immunized against diphtheria bacilli:

1. By treating first with slightly virulent and later with highly virulent cultures of diphtheria bacilli.

2. By the injection of diphtheria toxins in small quantities or toxins partially weakened by heat, and following with larger quantities. This is repeated with increasing doses.

3. By injection of serum from an animal immunized against diphtheria.

Also, in man, prophylactic injection of immune serum has been employed when there was danger of diphtheria, in part with very good results. See, for example, Slawyk (C. B. XXIV, 396). Regarding the almost universally acknowledged success of the antitoxin injection for therapeutic purposes in cases of disease, it is not necessary to enter into details here.

Special Diagnosis of the Coryn. Diphtheriæ.[1]—From the suspected material the following smear preparations are made:

1. Staining with methylene-blue or dilute fuchsin with a little warmth.

2. A preparation stained by Gram's method often presents the diphtheria bacilli more plainly, since the contaminating bacteria are in part unstained.

3. Granule staining by Neisser's method.

If there are found, in this way, abundant and especially long forms stained in segments with characteristic cross arrangement and many granules, then the diagnosis of diphtheria is to be considered as very probable.

To render the diagnosis more secure, delicate smear inoculations are made upon ascites-agar, by drawing the needle five or six times in succession over fresh parts of the nutrient medium. The cultures thus obtained correspond either to the typical picture of the diphtheria bacillus, with its growth of moderate intensity, or we obtain meager "xerosis-like" or luxuriant "pseudodiphtheria-like" cultures.

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[1] Bruno (Berl. klin. Wochenschr., 1898, 1127) attempted to make use of serum diagnosis here also. Diphtheria serum produced agglutination of certain diphtheria cultures, but not all. It was not sufficient to separate diphtheria and pseudodiphtheria.

They are then tested further as follows:

4. Staining of granules in twenty hours' serum cultures according to the method of Neisser. This is very highly recommended by C. Fränkel (Berl. klin. Wochenschr., 1897, 1087).

5. Titration of the acid formed in 5 c.c. of nonsaccharine bouillon in twenty and forty hours. If not less than 0.7 and not more than 1.2–1.5 c.c. of 1:40 normal alkali solution is required for neutralization, this speaks in favor of diphtheria. It is recommended that a parallel observation be made with known diphtheria bacilli in order to see whether a casual absence of acid production does not depend upon the constitution of the bouillon. (See p. 395.)

6. Animal experiment: If the injection of 1 c.c. of a twenty-four hours' bouillon culture produces the characteristic symptoms and death in about forty-eight hours (see p. 399), the diagnosis of diphtheria appears certain. With lessened virulence only slight local symptoms occur, and eventually only death from marasmus. (See p. 404.)

7. Demonstration of the protective action of antitoxin against the infection in especially difficult or uncertain cases.

By following this scheme, a typical diphtheria bacillus is easily diagnosed, usually only the means given in 1 to 4, and perhaps also 5, are required.

However, there occur in the mouth in cases of diphtheria, besides diphtheria bacilli which are typical in every way, also most numerous variations. See, especially Kurth (Z. H. xxviii, 409).

1. Non-virulent D. B., typical in all morphologic and biologic peculiarities. Kurth found 3 non-virulent out of 39 typical cultures.

2. Virulent D. B., typical in everything, except that they exhibit no granule staining (Neisser found 3 without granules out of 39 typical cultures). We found a form with very slight production of granules. This group passes over into the following.

3. Virulent D. B., typical in every way, but without the usual acid production. We found 1 out of 4 cultures examined.

4. Virulent D. B., typical in every way, but with very little tendency to the formation of longer forms.

5. Virulent D. B., typical in every way, but with such luxuriant growths upon glycerin-agar and potato that they cannot be distinguished macroscopically from the Corynebacterium pseudodiphtheriticum.

In other words, we speak of a true diphtheria bacillus whenever a bacillus stains in segments and presents a distinct, specific pathogenic action, without taking much account as to whether it corresponds exactly in one of the peculiarities of length, granule staining, appearance of cultures, and production of acid as given in the scheme for the diphtheria bacillus. Even if several of these peculiarities are found to differ from those in the scheme for the diphtheria bacillus, still a typically pathogenic organism remains for us a Corynebacterium diphtheriæ, for clinicians have formed this species, and the single pathogenic property appears so characteristic that we may build a differential diagnosis upon it alone.

It is much more difficult, if pathogenesis fails, to pronounce regarding the relationship to the true diphtheria bacillus. If all the morphologic and biologic peculiarities are present which belong to the true diphtheria bacillus, and the pathogenic property only is lacking, then it is safe to decide that one is dealing with a non-virulent, true diphtheria bacillus.

It is more uncertain if, besides the virulence, still other peculiarities fail; for example, the production of acid. Here the decision is doubtful, and the uncertainty increases the more peculiarities are simultaneously lacking —the more the organism approaches what are now customarily called "bacteria resembling diphtheria." We have devoted the following section to these.

The Pseudodiphtheria Bacilli of Writers.

Organisms resembling those of diphtheria, but not virulent, are found in great numbers in the mouths of diphtheritic and healthy persons, in the conjunctival sacs of healthy and diseased eyes, etc.[1] Proof has not been furnished that these organisms, which in their extreme forms differ widely from the diphtheria bacillus, are connected with it genetically. Therefore there are no essential reasons for regarding these forms simply as atypical diphtheria bacilli in the broader sense.

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[1] Schütz very frequently found in the sputum in tuberculosis, bacilli resembling those of diphtheria (Berl. klin. Wochenschr., 1898,

On the other hand, it is not possible to separate them naturally into definite varieties by the side of diphtheria bacilli, any more than this is possible in the forms of the Bact. coli and water vibrios. It is customary at present to dispose of this by designating the luxuriantly, succulently, and rapidly growing non-virulent forms as Corynebacterium pseudodiphtheriticum (Hofmann-Wellenhof) Lehm. and Neum., the scantily and delicately growing forms as Corynebacterium xerosis (Neisser) Lehm. and Neum., and the other non-virulent forms[1] are pressed into this scheme as well as possible.

Corynebacterium pseudodiphtheriticum. (Löffler.) L. and N.

(Plates 58-60, in part.)

Pseudodiphtheria bacillus of Löffler. Discovered by von Hofmann-Wellenhof in 1887. Described in detail by Escherich (Aetiol. der epid. Diphth.), Zarniko (C. B. VI, 153), and Prochaska (Z. H. XXIV, 373).

Rods, which upon serum are shorter and thicker than true diphtheria bacilli, show less often a tendency to form clubs and segments, but have a tendency to parallel grouping and are not virulent for guinea-pigs (Escherich). Upon glycerin-agar it grows not alone upon the inoculation line, but in two to four days spreads out over the surface of the agar. It varies from milky white to dirty yellowish or gray, is succulent, and the border is slightly notched (58, III).

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297). R. O. Neumann (not yet published) found in every case of catarrhal cold, but also in every healthy nose, often very abundant diphtheria-like organisms, sometimes growing luxuriantly, sometimes delicately, mostly producing a little acid and giving only slight granule staining. The virulence has not been investigated.

[1] These forms are usually not entirely non-virulent. C. Fränkel and others have seen animals die with marasmus a long time after the injection of large doses of bouillon culture.

The glycerin-agar plates appear correspondingly luxuriant (58, VIII, a); when magnified sixty times, they present dense, granular, dark colonies with ragged borders and opaque centers (59, IV, *a* and *b*). Upon potato, fairly abundant white growth. It is dry, elevated, lobulated, often resembling the species of mycobacterium and actinomyces (58, X). In bouillon the acid production (expressed in 1 : 40 normal alkali to 5 c.c. of bouillon), according to all writers, is usually very slight (*i. e.*, upon ordinary bouillon after twenty hours, 0.3–0.7; after forty hours, not more than 1.2; upon sugar bouillon after twenty hours, 0.6–1.4; after forty hours as much as 1.3 to 2.1), or there is none at all. After two to four days the alkalinity increases perceptibly. We have observed cultures, nevertheless, which produced upon sugar bouillon in forty hours as much as 3.2.

Old agar tubes often exhibit a brownish-red to brownish-black discoloration.[1] This phenomenon is inconstant, but, according to Escherich, is never present in diphtheria bacteria.[2] Upon gelatin there occurs a luxuriant growth, even at 18°; bouillon shows a more rapidly forming turbidity and a denser and later forming sediment than occurs with diphtheria bacteria.

In Gratz, v. Hofmann found this organism so frequently (26 times out of 45 healthy persons) in the mouth that he considered it a normal inhabitant of the mouth. Other writers found it much more infrequently. Escherich never found it in healthy persons in Gratz, twice in 100 cases of diphtheria, and ten times in association with other diseases of the throat. In Würzburg we found it not infrequently in healthy and diseased eyes and noses. Escherich admits the possibility that this organism may sometimes be recognized as a form or descendant of the diphtheria bacterium, yet it was impossible by the employment of most various means to render it virulent, not even by the simultaneous injection of streptococci.

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[1] An organism, obtained from Honl, of Prague, was very similar in every particular (staining, clubbing, branching, luxuriant growth, staining well by Gram's method), but presented a reddish tint in all cultures, especially intensely (rose) developed in the surface layer of a milk culture. We have cultivated a similar one from the nose with a luxuriant brownish-yellow growth (58, V).

[2] We obtained a brownish color of glycerin-ascites-agar in one of our cultures in ten to fourteen days, and in three others after a longer time (six weeks). For the cultures we are indebted to Dr. Silberschmidt (Zürich).

Important, but lacking confirmation, is the statement of Hewlett and Knight that they have succeeded in the London Institute of Preventive Medicine in converting the Hofmann-Wellenhof organism into the virulent diphtheria bacterium by passage through animals, and that typical virulent diphtheria bacteria may be changed into the typical Hofmann-Wellenhof organisms by careful heating (C. B. XXIII, 793).

Corynebacterium xerosis. (Neisser and Kuschbert.) L. and N.

(Plates 58–60 in part.)

Xerosis bacillus of Neisser and Kuschbert.

Grows especially in short forms, yet Heinersdorff, for example, represents a number which differ in no way from diphtheria bacteria, and we have also often observed such forms. According to all writers, the growth on Löffler's serum is dry and more scanty than that of the diphtheria bacterium, and still slower upon glycerin-agar (58, VIII, *c*). Upon potato no growth is to be seen. When magnified sixty times, it is not distinguishable from feebly growing forms of Coryn. diphtheriæ (60, VIII). When grown on Löffler's serum at 35° for nine to twenty-four hours, there are none, or only a few, of Neisser's granules.[1] Bouillon always remains clear, and acid production usually is absent: *i. e.*, at most, 0.6 in twenty hours, about 1.0 in forty hours; in sugar bouillon in twenty hours, 0.6–1.6; in forty hours usually only 1–1.5, but may be as much as 3.2. In numerous cultures from the eye and nose we usually observed slight acid production parallel with the limited growth, but sometimes, in spite of this, well-marked granule staining. Pathogenesis is lacking, according to all writers, and the organism appears to only accompany and not cause the xerosis processes in the eye which are accompanied by atrophy of the conjunctiva. The statement of Spronk (Deut. med. Wochenschr., 1896, 571) that a differentiation from diphtheria bacteria is possible from the absence of effect of diphtheria antitoxin against the Coryn. xerosis is doubted by most authors, especially since no pathogenic action is observed in the latter (for example, Heinersdorff, Archiv. f. Oph., Bd. 46, p. 1).

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[1] Not infrequently we found distinct granule staining, also in nonvirulent cultures which produced no acid and whose growths were dry and scanty.

Kurth found in one-fifth of true diphtheria cases forms, probably belonging here, which were absolutely non-virulent (Bac. pseudodiphtheriticus alkalifaciens, Kurth), and also three forms which produced acid as actively as the true diphtheria bacteria (Bac. pseudodiphtheriticus acidumfaciens). Gelpke (see below) found in his cultures (in all?) less acid production in ordinary bouillon, nevertheless much greater initial acid production in grape-sugar bouillon than with diphtheria bacteria.

Gelpke (Bact. septatum, etc., Karlsruhe, 1898) has recently regularly isolated an organism as the cause of "catarrhal swelling," which is a specific inflammation of the eye characterized especially by bluish-red discoloration and swelling of the fold of the conjunctiva, formation of a fibrinous exudate, great pain and photophobia, together with general symptoms. He has called the organism Bacterium septatum Gelpke, considering it a new variety, in spite of great similarity to the short xerosis forms. So far as we see, aside from a not very great pathogenic effect upon the human conjunctiva, as demonstrated by Gelpke in some cases, there is nothing in the exhaustive description of the organism to distinguish it from the Coryn. xerosis. Other authors appear to have obtained the same impression.

What we have said in the preceding pages shows that, as in the case of the virulent ("true") diphtheria bacteria, so also in the non-virulent, there is a long series of exceedingly closely related forms, which may be differentiated by varying combinations of characteristics,—luxuriance, length of rods, granule formation, production of acid, etc.,—and which form a gradational series, into which the true diphtheria bacterium also fits.

This is rendered still clearer in the following brief tabulation of a part of our findings:

	Variety and Source of Culture.	Microscopic Picture from Glycerin-agar.	Pathogenic Effects.	Glyc.-agar Plate Culture, Macroscopically.
1.	Corynebacterium diphtheriæ from exudate in throat (typical diphtheria).	Slender rods, clubbed at both ends. Branching. Segmentation distinct; among them also shorter organisms. Typical diphtheria.	Guinea-pigs injected subcutaneously die after twenty-four hours.	Whitish-yellow, luxuriant, moist.
2.	Corynebacterium diphtheriæ from exudate in throat (atypical diphtheria).	Much more luxuriant and thicker. The swellings are more irregular. Many short, thick, wedge-shaped organisms. Segmentation. Branching.	Guinea-pigs injected subcutaneously die after forty-eight hours.	Grayish-white, delicate, transparent.
3.	Corynebacterium pseudodiphtherit. from the eye.	Small, thick, wedge-shaped organisms, often in pairs. Part in form of oval cocci. Segmentation in the middle. No branching.	Not pathogenic.	Grayish-white, delicate, transparent; later more luxuriant.
4.	Corynebacterium pseudodiphtherit. from the nose.	Longer rods, clubbing more to one side. Segmentation present. Among them many short forms. No branching.	Not pathogenic.	Yellowish, luxuriant, succulent; later yellowish-brown.
5.	Corynebacterium xerosis from the nose.	Almost without exception small rods pointed at the ends, arranged in pairs. More rarely thick forms with swellings. No branching.	Guinea-pigs not killed by 5 c.c. bouillon injected intraperitoneally.	Grayish-white, delicate, transparent.
6.	Corynebacterium xerosis from the eye.	Regularly segmented slender forms with clubbed swellings. Very often also shorter, wedge-shaped rods. No branching.	Infiltration at point of injection in guinea-pigs (subcutaneous), but do not die.	Whitish, dry, a little more luxuriant than preceding variety.

Plate Cultures, Glycerin-agar, Microscopic.	**Bouillon Culture.**	**Granule Formation on Serum Media.**	**Production of $\frac{1}{10}$ Normal Acid.**			
			In 5 c.c. ordinary bouillon.		**In 5 c.c. sugar bouillon.**	
			After 20 hrs.	**After 40 hrs.**	**After 20 hrs.**	**After 40 hrs.**
Grayish-brown; fairly transparent; splintery appearance; torn, ragged edge	Slightly cloudy. Abundant, sandy precipitate.	Detached granules at the poles. Atypical.	0.6	1.7	0.6	5.7[1]
Like No. 1, but thicker and only slightly transparent.	Very cloudy. Sediment homogeneous, slight, easily distributed.	Regularly at the poles, numerous, also detached ones in the middle.	1.2	2.3	3.8	5.8
Like No. 1.	Almost clear. Sediment slimy, easily distributed.	Detached granules.	0.5	1.2	0.5	1.3
Like No. 2. Interior very granular. The periphery resembling sarcinæ.	Clear. Sediment granular, abundant, easily distributed.	Granules very irregularly distributed, not very few.	0.9	0.5	2.5	3.2
Like No. 1.	Like No. 2.	No granule staining.	0.6	1.1	0.5	0.7
Like No. 2.	Like No. 3.	Here and there a polar granule, also isolated ones in the middle of rods.	1.0	1.0	1.3	2.1

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[1] At first exceedingly slow acid production!

Supplementarily, we may here mention the following:

Bacillus pseudotuberculosis ovis. (Preisz.)

The rods are smaller and finer than diphtheria bacteria and stain well by Gram's method. Grows only at incubator temperature, and upon agar and serum only scantily and dry. Upon bovine serum there is often a striking orange-yellow color. Cultivated from the kidney of a sheep. Injected intravenously into rabbits and guinea-pigs, it produces pseudotuberculosis (A. P., 1894, 231).

Bacillus pseudotuberculosis murium. (Kutscher.)

Similar in many points to the preceding, but pathogenic for mice only. Cultivated from the lung of a diseased mouse (Z. H. XVIII, 327).

The interesting "sporogenic" pseudodiphtheria bacillus of De Simoni (C. B. XXIV, 294) scarcely seems to belong here, in spite of certain similarities between it and the diphtheria bacillus (striped rods).

2. Mycobacterium Lehm. and Neum.

Cultures upon solid nutrient medium are elevated, more or less wrinkled and dry. Microscopically: thin, slender rods, often with typical dichotomous branching, sometimes forming unbranched or branched threads. When the rods have been stained with hot carbol-fuchsin, they give up the stain from the action of acids with great difficulty; they are "acid proof"—*i. e.*, they behave toward stains much like the spores of ordinary bacilli.

Mycobacterium tuberculosis. (R. Koch.) L. and N.

(Plate 61.)

Synonyms.—Bacillus tuberculosis R. Koch. Bacillus Kochii Aut. nonnull. Sclerothrix Kochii Metschnikoff (V. A. CXIII, 70). See page 128.

Common Name.—Tubercle bacilli.[1] T. B.

Most Important Literature.—R. Koch (Mitt. aus. d. Gesundheitsamt II, 1884); Nocard and Roux (A. P. I, 19); Czaplewsky, Untersuchung des Auswurfs auf Tuberkelbacillen, Jena, 1891; Fischel, Morphologie

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[1] In the following we more often employ the common name of tubercle bacillus (T. B.), but in spite of this we do not consider the further application of the scientific name, Bacillus tuberculosis Koch, to be proper.

und Biologie des Tuberkuloseerregers, Vienna, 1893; Coppen Jones (C. B. XVII, 1); Hayo Bruns (C. B. XVII, 817); Cornet, Die Tuberkulose, 1899.

Microscopic Appearance.—In sputum and cultures usually unbranched, slender rods, 1.5–4 μ long, only 0.4 μ thick, which often are slightly bent (61, VII, IX, X).

More recently many writers have observed thread and true branched forms—in sputum and in cultures, and in the latter, with careful preparation, they are predominant—which are injured and broken apart by only the roughest preparation. (Literature, history, and good illustrations by Coppen Jones, *l. c.*) Lubinski obtained long threads without branching upon acid potatoes (C. B. XVIII, 125).

Inside of the tubercle bacillus from sputum and pure culture there are sometimes found unstained vacuoles, sometimes peculiar structures which give an especially intense, dark red color with carbol-fuchsin. Still, these latter bodies do not present the regular form of the true spores of bacilli; also statements regarding resistance and germination are not at hand. Coppen Jones compares them to the chlamydospores of the mucorini.

In the same article the same author described very remarkable forms from tubercular sputum resembling the clubs of actinomyces, but which he recognized not as organized forms directly formed by the T. B., but (like actinomyces-clubs) as secretions, concrements, etc.

Friedrich found T. B. resembling actinomyces—*i. e.*, clubbed, dense, radiating formations—in sections of organs from animals which succumbed to a rapid tuberculosis infection (see p. 416).

Motility.—According to all authors motility is lacking. Schumowski claims to have constantly seen a slow motion of the T. B. (C. B. XXIII, 838.)

Staining Properties.—The T. B. stains so difficultly and imperfectly with the ordinary aqueous solutions of anilin dyes that these are never employed. Also the stain suggested by Koch, accomplished by prolonged action of alkaline methylene-blue, has only a historical interest.

To-day two methods (Tech. Appendix), with innumerable (insignificant) modifications, are almost exclusively employed. Of these, we always use that of Ziehl-Neelsen.

Also, Gram's method is successful, but is not especially recommended, since it does not possess the advantage of a specific reaction.

Relation to Oxygen.—Without oxygen, no growth.

Requirements as to Temperature and Reaction of Nutrient Media.—Growth occurs between 29° and 42°, the optimum being 37°. Under all circumstances growth is slow.

Preliminary Remarks Concerning Cultures. — Upon the ordinary agar and gelatin nutrient media the T. B. grows scantily or not at all. For its cultivation, besides solidified blood-serum, glycerin-agar is almost exclusively employed (Nocard and Roux, C. B. I, 404).

Glycerin-agar Plate.—Surface colonies like those on the glycerin-agar streak.

Glycerin-agar Streak. — At first there are minute, crumbly growths, irregular in form, white to yellowish-white, fairly elevated, devoid of luster or faintly glistening (61, I). Later, after three to four weeks, the colonies grow out and have lobulated sinuate borders. The peripheral portions are still thinly transparent, and at intervals there are formed elevations, like mountain ranges, running from the border toward the center, which gradually converge to form a mountain stem in the middle. The elevations are usually yellowish to brownish in color; the depressions, whitish to grayish-yellow. Still later the entire colony becomes brownish (61, II). We once obtained an orange discoloration. Hüppe reports that he has grown cultures which presented a pronounced yellow to reddish-yellow color. (See p. 430.) Kitasato cultivated a luxuriantly growing variety of Myc. tuberculosis. (Compare Myc. tub. avium, p. 418.)

Blood-serum Streak.—A slight growth in the form of light-colored, dry, crumbly scales becomes visible microscopically after about six days and macroscopically after ten to fourteen days. Blood-serum is never liquefied. When magnified sixty times, the colonies, especially at the borders, present S-shaped flourishes consisting of nothing but parallelly arranged rods (61, V).

Potato.—If potato is inclosed in an air-proof (*i. e.*, protected from evaporation) reagent glass, there slowly develops small, crumbly, yellowish, friable masses, devoid of coherence, much elevated above the surface of the potato, dull or with a faint luster (61, III). The culture is well developed after about three weeks. (See Pawlowsky, C. B. IV, 340). The growth is better if air can enter and other precautions are taken to prevent drying of the potato.

Fluid Nutrient Media.—If glycerin (up to about 4%) is added to the nutrient fluids, the T. B. will grow very well upon most various mixtures; for example, bouillon, potato water, and artificial non-albuminous nutrient media. As an example of such a medium we may mention: Mannite, 0.6; citrate of magnesium, 0.25; sulphate of ammonium, 0.2; glycerin, 1.5; diphosphate of potassium, 0.5. See Proskauer and Beck (C. B. XVI, 974).

According to Rabinowitsch, the T. B. forms a thick film upon all liquid nutrient media and gives off an odor of flowers. Formation of endogenous spores does not occur, and whether a form of arthrospore is produced is at least very doubtful. (See p. 411 regarding chlamydospores.)

Resisting Powers Against:

(*a*) *Light:* Pure cultures are very susceptible to direct sunlight; are also injured by pale, diffuse daylight (according to Koch, cultures on a window die in five to seven days).

(*b*) *Drying:* According to Sawitzky (C. B. XI, 153), human phthisical sputum retains its virulence, when dried at room temperature, for two and one-half months; also sunlight does not here produce injury. Obici (C. B. XIX, 314) obtained a series of similar results. On the contrary, Migneco found them dead in the sun after twenty-four to thirty hours if the dried sputum was not in too thick a layer (A. H. XXV, 361). Tubercule bacilli dried on cigars die in ten days; on the contrary, on paper they may live as long as four weeks.

(*c*) *Moist heat:* 50° does not kill in twelve hours, 55° kills in four hours, 60° in forty-five to sixty minutes, 70° in ten minutes, 80° and 90° in about five minutes, 95° in one minute (Forster, H. R. II, p. 869).

(*d*) *Cold:* It is borne very well; for example, winter cold for twenty-one days by bouillon cultures.

(*e*) *Disinfectants:* Injure slowly, especially T. B. which are found in sputum; 3% carbolic acid, for example, kills T. B. only after twenty hours.

An extensive survey of the tenacity of the T. B. is given by Schneiderlin, Dis. med., Freiburg, 1897.

Chemical Activities.—(*a*) No chromogenesis or production of odoriferous substances.

(*b*) Cellulose is formed in distinction to many other investigated bacilli.

(*c*) Indol and H_2S production were not observed in our cultures.

(*d*) Regarding toxins, see page 417.

Distribution.—(*a*) *Outside the body:* So far, found only in living rooms (dust of railroad cars, street dust, etc.) in places where tubercular cases have deposited their sputum. In the air they are seldom found, and then are isolated.

They are very frequently found in milk. A third of tubercular cows furnish, even when the udder is healthy, milk containing T. B. Still there is great variation. While the butter of a large Berlin dealer contained T. B. in the butter in 100% of the cases (Obermüller, H. R., 1897, 712), thirteen other establishments in Berlin were proved to furnish butter free from T. B. (Rabinowitsch, C. B. xxv, 77).

(*b*) *In the healthy body:* Very many apparently healthy individuals, men and animals (cows), present at autopsy smaller or larger, often completely healed tubercular foci. Of such men there are said to be 66% with latent or healed tubercular foci; and of these, it is the principal disease in 53%, a secondary affection in 6%, and entirely latent in 41% (Schlenker). Healthy nurses and physicians of tubercular patients are said to often show T. B. in the nasal mucus.

(*c*) *In diseased human organism:*[1] It occurs as the exclusive and essential cause of miliary tuberculosis, of bone, gland, and joint tuberculosis (caries, fungous inflammation, white swelling, etc.), of lupus (tuberculosis of the skin), of intestinal, peritoneal, renal, and meningeal tuberculosis, of dry and serous pleuritis,[1] etc. All the organs may be affected with tuberculous disease.

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[1] Regarding cases of men affected with fowl tuberculosis, see page

Part of the tuberculous affections of the lungs are dependent upon the T. B. alone; in phthisis, streptococci play a very important secondary rôle as the cause of the typical irregular temperature curve and as destroyers of the pulmonary tissue with suppuration. "Anatomical" tubercles are only in part caused by the T. B.

The port of entry of the T. B. may be in any part of the body (lung, intestine, skin, wounds of the skin), and is said by many authors to be located especially often in the tonsils.

Tuberculous mothers sometimes furnish tuberculous ova, or tuberculous fetuses (eventually through placental tuberculosis); tubercular fathers, even with tuberculosis of the testicle, scarcely ever transmit T. B., but certainly do a disposition to tuberculosis (Gärtner, Z. H., XIII, 101). In the same place are also given many statements from the literature.

(*d*) *In animals:* Tuberculosis is very frequent in cows ("Perlsucht"). In newly born calves tuberculosis (always miliary tuberculosis) is a rarity (according to Klepp, with exhaustive examination, it occurs in about 3% of slaughtered calves!). In slaughtered cattle as high as 35% have been found to be tuberculous; in old milk cows, as high as 80%.

In places tuberculosis occurs frequently also in swine,—for example, in the slaughter-houses of Dantzic in 11%,—yet mistakes in connection with the necrotic areas of swine plague ("Schweineseuche") have been observed. Sheep, goats, horses, dogs, and cats sometimes, though rarely, present very extensive tuberculosis. Rabbits and guinea-pigs sometimes present tuberculosis rather frequently; yet of 3000 guinea-pigs which were killed during 1890–96 in the Department of Health of Berlin, not a single instance of spontaneous tuberculosis was observed[2] (Petri).

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[1] Pleural exudates, apparently free of bacteria, are very often of a tuberculous nature.

[2] Vagedes has isolated twenty-eight different cultures of tubercle bacilli from man and two from animals ("Perlsucht"), mostly from

Experimental Observations Regarding Pathogenic Effects.—(*a*) *In animals:* With T. B. from man it is very easy to infect cattle, swine, horses, and especially monkeys and guinea-pigs; also dogs are easily infected, especially intravenously. Fowls are immune; in hens, at most, there occurs a small, local area from inoculation in the comb.

Infection follows the introduction of T. B. by all sorts of methods (also inhalation and feeding), but most certainly by the intraperitoneal. At the place of infection a caseous area is formed, and in the neighborhood (omentum, peritoneum) an acute miliary tuberculosis. With intravenous infection a general miliary tuberculosis develops. Tubercle bacilli, attenuated by iodoform, cause in rabbits, sometimes the picture of chronic phthisis in man, sometimes the typical pearly disease ("Perlsucht") (Troje and Tangl, C. B. XI, 613).

If rabbits are injected subdurally or into the kidneys,—according to Friedrich, also into the veins,—then areas are often produced which in from fourteen to fifty days correspond throughout to pictures of actinomyces: *i. e.*, a central tangle of genuine branching threads, limited at the periphery by clubs. The central structure is acid proof; the clubs are often only feebly so, and sometimes are stained blue with a counterstain of methylene-blue. Both the threads and clubs stain well by the Gram-Weigert method, while in the actinomyces the clubs rarely retain the stain in Gram's method.

The close relationship between tuberculosis and actinomycosis is constantly demonstrated by these investigations. Details will be found in the literature cited above. For the latest researches, with beautiful illustrations, consult Schulze and Lubarsch (Z. H. XXXI, 153 and 187). In the same place special staining methods are also described.

(*b*) *In man:* Experimental tests are lacking. Of the clinical experiences, some cases of disease following infec-

the pus of cavities and pulmonary nodules. The virulence for animals proved to vary very much. If a culture was highly virulent for rabbits, it made no difference whether an infection was produced in the eye or subcutaneously or intravenously, and such cultures were also always very virulent for rats.

tion of a wound of the hand by sputum (injury by a broken sputum glass) have the force of experimental demonstration.

Toxins, Immunity, Immunization.—From old cultures of the T. B. upon glycerin bouillon by means of evaporation and precipitation with alcohol, an albuminous body is obtained, formerly known as "tuberculin," and now as "old tuberculin." When this is injected in cases of tuberculosis (Koch), it exerts a peculiar influence upon the tuberculous process. Very weak doses call forth a moderate increase of inflammation at the seat of the tuberculous disease, with fever, while healthy persons exhibit neither fever nor noticeable local symptoms. As pointed out by Buchner and Römer, the proteins of other bacteria have an exactly similar effect upon tuberculosis.

While Koch and some of his students obtained good, or at least satisfactory, curative and immunizing results with the old tuberculin in man and animals, most investigators, after a brief enthusiasm, abandoned the preparation as very rarely useful, but also as very often injurious. Then Koch sought to improve his preparation, and, under the name of "Tuberculin TR," recommended a new preparation, prepared as follows:

Virulent T. B. are dried and then pulverized, suspended in water, again pulverized, and then separated by centrifugation into a sediment, and a supernatant fluid. The latter is decanted and only the further aqueous extract is employed, which is obtained by pulverizing and by separation of the solid ingredients by centrifugation (Deut. med. Wochenschr., 1897, 209).

H. Buchner, following the method of E. Buchner, has obtained a "tuberculoplasmine" by trituration and compression of fresh tubercle bacilli, concerning which no practical results appear to have been published.

Koch has completely immunized a series of guinea-pigs with his TR by means of carefully but actively increasing doses. Complete immunity was obtained about two or three weeks after the administration of large doses. Also Koch has obtained a cure in previously infected guinea-pigs, but the treatment must be instituted not later than eight to fourteen days after infection, because of the rapid course of the disease in guinea-pigs. Also the absorption of tuberculin in animals already infected is slower, and therefore it acts more unsatisfactorily. It must not be disguised, however, that Baumgarten and others arrived at absolutely negative results with the new tuberculin in guinea-pigs, as previously was the case with the old tuberculin (C. B. xxiii, 587); small doses were worthless, and the larger the doses, the greater the disappointment. Regarding the value of the new preparation in man, there is no unanimity. Spengler (C. B. xxiii, 523) gives a favorable judgment, but unfavorable or skeptical opinions are in the majority. See, for example, Stempel (Münch. med. Wochenschr., 1897, No. 48) and Bukovsky (C. B. xxiii, 518). A review of collected literature is furnished by Bussenius (C. B. xxii, 621). Maragliano claimed to obtain good results with a tuberculosis serum, but his results have been but little verified from other sources (at least such is the experience of Hager, Münch. med. Wochenschr., 1897, 853). Also Behring recently hoped to succeed in reestablishing an antitoxic tuberculosis serum (Congress of Hygiene, Madrid, 1898). Tuberculin now plays a large part, entirely aside from its therapeutic use, as a diagnostic aid in tuberculosis. For details, see page 437. For the differential diagnosis of the T. B., see page 436.

Mycobacterium tuberculosis β avium. (Maffucci.) Lehm. and Neum.

Synonym.—Bacillus tuberculosis avium Maffucci.
Common Name.—Bacillus of fowl tuberculosis.

Most Important Literature.—Maffucci (Z. H. xi, 445); Straus and Gamaleia (C. B. x, 300); Courmont (C. B. xiv, 602); Kruse (C. B. xv, 50 1); Pfander (histologic, C. B. xii, 264); Fischel (Untersuchungen über die Morph. und Biol. des. T.-Erregers, Wien, 1893).

From the standpoint of our present knowledge this interesting organism, first separated from the T. B. by Maffucci, must be looked upon as only a form of the latter which has become acclimated to the bird's body (its high temperature), but which is occasionally also pathogenic for other creatures.

In favor of this are the observations of Kruse and Pansini. From a guinea-pig, inoculated with the juice from tuberculous organs of cattle, and from another infected with human sputum, typical fowl tubercle bacilli were cultivated which were pathogenic for chickens.

In accord with this, Johne and Frothingham found fowl tuberculosis in cattle (C. B. XIX, 564) and Nocard in horses (C. B. XXI, 807).

After prolonged cultivation on artificial nutrient media at ordinary incubator temperature, the bacillus of fowl tuberculosis becomes pathogenic also for mammals (Courmont, C. B. XIV, 602). Fischel (C. B. XIV, 632) observed transition forms connecting the two diseases. Finally, Cadiot, Gilbert, and Roger were able to invert the pathogenic properties of both varieties by continued transfer (C. B. XIX, 567).

The following are given as the points upon which the differential diagnosis of the avian form rests.

Microscopic Appearance.—Like the T. B., sometimes a little longer and slimmer. Staining properties are the same.

Requirements as to Nutrient Media.—Does not grow on potato; otherwise the same as the T. B.

Requirements as to Temperature.—Limits, 35°–45°. In contradistinction to the T. B., it grows very well and without reduction of virulence at 43° (Straus and Gamaleia). The T. B. generally does not grow above 42°.

Serum and Agar Cultures.—They are always softer, more succulent, more luxuriant, and grow more on a level. However, Kruse has observed a dry culture, also cultures which, upon certain varieties of agar, take on a reddish, blackish, violet color.

Fluid Nutrient Media.—The pellicle is not so firm as in the T. B.

Cultures live for two years.

Pathogenic for birds[1] when introduced in every way except by feeding, attacking especially the liver and spleen. Also the embryos of chickens in incubated eggs can be infected. The course is very chronic; giant cells are rare; bacilli are present in great abundance.

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[1] The very common tuberculosis of parrots is usually produced by true T. B., the animals being infected by receiving food from the mouths of tuberculous persons.

Immunity against avian tuberculosis exists in the dog, monkey, and guinea-pig; but the last-named animal after injection dies with a slow marasmus (chronic intoxication). Rabbits are rarely susceptible.

Leray (H. R., 1896, 358) describes differences in the pathologico-anatomic picture in rabbits which are inoculated with avian and mammalian tuberculosis. In the former caseation is lacking, but there are many giant cells, with common inclusions of the organisms; in mammalian tuberculosis the reverse obtains, the caseation being extensive, giant cells few, the T. B. usually free.

Mycobacterium tuberculosis var. γ piscicola. L. and N.

(Plate 62, v–ix.)

Literature.—Bataillon, Dubard, and Terre (C. B. xxii, 61); Král and Dubard (Rév. de la tuberc., 1898, No. 2).

The French authors cultivated the organism, which resembles the T. B., from a tumor of carp about the size of a pigeon's egg. It is not motile, is acid proof, forms branches, and grows at 23°–25° as the optimum temperature (minimum, 12°). In bouillon there are abundant flocculi, which settle to the bottom; upon potato there occurs a thin whitish growth; gelatin is not liquefied.

In order to prove that their organism is the T. B., acclimated to cold-blooded animals, fish and frogs were inoculated and fed with cultures of human and avian tuberculosis. From the organs of such fish the var. piscicola was in fact obtained.

In association with Dr. Kumulis we have thoroughly studied a culture obtained from Král, and have completely confirmed the statements of the French investigators. Growth stops at once at 37°, and at 20°–25° it is a little more luxuriant than that of the ordinary T. B. in the incubator. In the microscopic examination we saw no branching, probably by chance. In gelatin plates (62, vi) there occurs a rather tough, dry, whitish-yellow, wrinkled growth, which upon being magnified sixty times corresponds to the T. B. upon glycerin-agar (62, vii). In the gelatin stab the growth is scanty; upon the surface a dry, thin, wrinkled pellicle, with no liquefaction. Upon agar just like the T. B. on glycerin-agar, only a little more luxuriant and more thickly padded (62, V). Bouillon is clear with a crusty pellicle. The growth on potato is wrinkled, thick, sharply outlined, whitish-yellow. The growth upon milk is strikingly different from all varieties studied by us. It is not coagulated, and after one to three months takes on a dark violet-gray color, the pigment being soluble in alcohol.

Mycobacterium tuberculosis δ ranicola. L. and N.

The efforts of various investigators to acclimate the T. B. to the frog resulted in the information that also here the T. B. is gradually transformed into a variety which grows at lower temperatures. For details see Lubarsch (Z. H. XXXI, 187).

Mycobacterium tuberculosis ε anguicola. (Moëller.) L. and N.

Moëller, Therapeutische Monatshefte, Nov., 1898; Lubarsch (Z. H. XXXI, 187).

Isolated from the spleen of a blindworm which was infected one year previously with human tuberculosis (sputum). Grows best at 22° (generally not at all at 28° to 37°); upon agar it forms a moist, glistening white layer! In dilute bouillon and non-albuminous nutrient media plentiful branching occurs. It cannot be inoculated into rabbits.

Mycobacterium lepræ. (Armauer Hansen.) L. and N.

(Plate 62, I–IV.)

Common Name.—Lepra bacillus.

Principal Literature.—Max Wolters, Der Bacillus lepræ (C. B. XIII, 469), and Finger in Ergebn. der allg. Aetiologie, 1896. Compare also Mitteilungen und Verhandlungen der internation. Leprakonferenz in Berlin, Oct., 1897, 2 Bände; Babès: Der Leprabacillus and die Histologie der Lepra, Berlin, 1898.

Since the communications of Armauer Hansen and Neisser (Virch. Archiv, LXXXIV, 514) there has been no doubt that the cause of leprosy is a non-motile organism, very closely related to the tubercle bacillus. These organisms, which are often a little shorter than the T. B., are found in the specific new-formation (nodes and nodules) of leprosy in the various organs in cases of the disease, and often in enormous numbers. The bacilli are found in groups, the individuals being parallelly arranged, and lie within special "lepra cells," which have recently been explained, sometimes as confluent, proliferating lymph endothelium, sometimes as lymph thrombi.

By means of staining reactions the L. B. cannot be certainly differentiated from the T. B. They stain by the Koch-Ehrlich method just as well as the T. B., and, like them, are also stained by Gram's method and by a sufficient action of aqueous anilin dyes. A difference is said to consist in this, that the L. B. is so well stained in six or seven minutes with an aqueous solution of fuchsin that good preparations are obtained after washing with water, while the T. B. is not; on the contrary, alkaline methylene-blue stains the T. B. quicker than the L. B. Compare the controversies upon this between Baumgarten and Wesener (C. B. I, 450, 573; II, 131, 291).

Still, all authors are now agreed that the staining reaction cannot help much in the differential diagnosis, no more than the form of the bacilli,[1] from which it follows

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[1] The following difference in sections is given by Spiegel from Unna's laboratory (C. B. XXI, 817):

	LEPROSY.	TUBERCULOSIS.
Number of bacilli:	Exceedingly abundant in all organs and secretions.	Very much less numerous.
Arrangement of the bacilli:	In heaps like a cigar in form.	More as individuals or in irregular bunches.
Form:	Rod-shaped, straight, and plump.	Thready, curved, and fine.
Angles:	Sharp.	Roundish.
Appearance of granules:	Coarse.	Fine.
Arrangement of granules:	Widely separated.	Close together.

These differences are naturally never so typical as here appears.

that the separation of leprous and tuberculous affections in the cadaver appears often impossible, since at least it is made differently by different persons. Since, according to Hansen and Looft (Bibl. med., 1894), the cause of death in 40% of the cases of leprosy is tuberculosis, this uncertainty is very unfortunate.

While for a long time efforts to obtain cultures of the infective agent from cases of leprosy were frustrated, more recently positive results have multiplied.

Almost all the cultures obtained present branched forms closely related to the Myc. tuberculosis; the typical acid-resisting power was rarely present (Bordoni-Uffreduzzi, Z. H. III, 178, with plate); sometimes there was a certain limited acid-proof property, and at other times none at all. Babès, who, if not the first to study this subject, has studied it most extensively, holds the organism, for evident reasons, to be the cause of lepra in spite of the absence of acid-proof quality (C. B. XXV, 125).

The growth upon artificial nutrient media (glycerin-agar, glycerin-serum-agar, glycerin-potato) was found by all writers to usually be delicate and slow; morphologically and biologically their behavior was very similar to the T. B. Our culture obtained from Král exhibited a good, although a slow, growth. Morphologically the rods resemble the T. B., but also the diphtheria bacterium.[1]

An additional proof that we may recognize the cultivated organisms with the greatest probability as the Mycobacterium lepræ has been furnished by Spronck (C. B. XXV, 257). It is the demonstration of agglutination of the questionable organisms by serum from many cases of lepra, even in high dilution.

Animal experiments are said by some authors to have succeeded (see Wolters), but no one has produced typical leprous changes. The greatest number of writers observed a rapid death of the bacteria introduced, and ascribe the positive results of others to tuberculous infection.

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[1] Czaplewski (C. B. XXIII, 97), who has also isolated an organism belonging here, says with entire truth that these forms constitute a connection between the diphtheria and tuberculosis groups, or, according to our terminology, the genus Mycobacterium and the genus Corynebacterium, which, in the first edition of this book, we placed side by side as closely related. See also Levy (A. H. XXX, 168).

Because of the prolonged period of incubation, it is difficult to determine the way by which the infection in man gains entrance. Generally an infection through the various mucous membranes and slight wounds is assumed; there are said to be no ports of infection in the alimentary and respiratory organs. Congenital lepra is at least very rare. Abundant L. B. are found in the sperm and milk. They have never been found in the ovaries. Except in the secretion from ulcers, they are found most frequently in the nasal secretion (128 times in 153 cases) (Sticker). The nose is the most common location of the primary affection, as well as the most dangerous source of contamination of the surroundings (Sticker, Münch. med. Wochenschr., 1897, Nos. 1063 and 1103).

A positive differential diagnosis of isolated and cultivated lepra from tuberculosis organisms can at present only be based upon the less developed acid-proof quality, the more delicate growths, and perhaps the agglutination.

Organisms in Verrugas.

We may only mention briefly that in the Peruvian "Verruga," an endemic disease characterized by rather large cutaneous swellings (hypertrophy of the papillary bodies), and formerly much confused with syphilis, organisms have recently been found in all the organs which are very similar to the T. B. morphologically and tinctorially. See Letulle, Nicolle, Odriozola (C. B. xxiv, 889).

Mycobacterium smegmatis. L. and N.

Common Name.—Smegma bacillus.

Since the investigations of Tavel (C. B. i, 673) and Matterstock (Mitt. a. der mediz. Klinik Würzburg, ii) we have known that still another mycobacterium is found in the smegma of the prepuce and clitoris, and only recently it has been cultivated a few times.

Laser (Münch. med. Wochenschr., 1897, 1191) first succeeded in cultivating an acid-proof, non-motile bacillus, corresponding in morphology to the smegma bacillus.

Upon agar smeared with blood the growth was scanty, like streptococci; upon glycerin-agar, like dewdrops; upon the ordinary nutrient media it grows poorly or not at all. The organism was not pathogenic. Czaplewski (*eod. loco*) has observed a little better growth in a culture, and also yellow, honey-like growths on potatoes. See also Grünbaum (*eod. loco*, No. 45, p. 1254).

Stolz (A. H. xxx, 156) has described a very closely related, beautifully branching, but not especially acid-proof organism, which grows very poorly in cultures.

In practical medicine more interest attaches to the question whether the smegma bacillus can be differentiated from the T. B. microscopically from its staining properties, than to its cultivation. This delicate question, with which we have had no personal experience, has been the subject of very much investigation. According to Grethe (Fort. d. Med., 1896, No. 9), a method of Weichselbaum is to be especially recommended for differential diagnosis. The preparation is stained with hot carbol-fuchsin, thus staining the T. B. and smegma bacilli red; then a saturated alcoholic solution of methylene-blue is allowed to act upon it (how long is not stated), when gradually, even in the thickest parts of the preparation, the smegma bacilli become blue, the tubercle remaining red.

According to the careful studies of Bunge and Trautenroth, there are, as was to be expected, acid-proof smegma bacilli which are quite different from one another, but none of them retains the stain after the following process: Absolute alcohol, three hours; 5% chromic acid, fifteen minutes; carbol-fuchsin, two to three minutes; dilute sulphuric acid, two to three minutes; concentrated alcoholic solution of methylene-blue, at least five minutes. Honsell (C. B. xxi, 700) recommends for the same purpose: Ordinary carbol-fuchsin stain, ten minutes in acid alcohol (absolute alcohol 97 parts, HCl 3 parts), wash in water, washing in alcoholic methylene-blue diluted one-half. Only the T. B. remain red.

For differential diagnosis, after a preparation has been stained according to the usual method for T. B. with gentle action of acid, and acid-proof red rods are found, then a second preparation must be stained by one of these methods, and if blue rods are obtained, the smegma bacillus can be diagnosed. Finally, also cultures may be prepared upon ordinary nutrient media to search for Mycobacterium lacticola and related organisms.

Micro-organisms in Syphilis.

Lustgarten (Wien. med. Wochenschr., 1884, and Wien. Jahrbuch, 1885) was the first to find micro-organisms in syphilis by modern methods. He succeeded in staining, by means of a special method, organisms resembling the tubercle bacillus, in the interior of syphilitic gummas and in the primary lesions (Technical Appendix). Doutrelepond arrived at similar results.

After the discovery of very similar organisms in normal smegma (see p. 424) had very much reduced the significance of the syphilis bacillus in the secretion of the primary lesion, and after very many investigators had sought in vain for Lustgarten's organism in the interior of syphilitic bodies, it was the general opinion that Lustgarten's positive findings in gummas were to be explained by a mixed infection with tuberculosis.

After ten years scarcely more than one article worthy of notice upon the etiology of syphilis had appeared, when van Niessen undertook to investigate anew this important question. Unfortunately, he entered upon the work with absolutely insufficient knowledge, and his first publications (C. B. XXIII, 48) deserve in general no serious discussion, as they were so full of technical errors.

Van Niessen has recently made further communications (Wiener mediz. Presse, 1899, Nos. 11–18) of investigations, carried out in the St. Petersburg institute of Prince Oldenburg, which indeed show that he has learned much in the meantime, but which can in no way be accepted as proof for his assertions. Reexaminations by known investigators are absolutely essential.

Van Niessen succeeded in cultivating an organism, which resembles somewhat the Corynebacterium diphtheriæ, from the blood of suitable cases of secondary syphilis, and also from flat condylomas which were not ulcerated (how often is not clear). It exhibits distinct clubbing and branching, stains with the ordinary anilin dyes, and also well by Gram's method, and often (not always) by Lustgarten's, but not by Ziel-Neelsen's method. The statements regarding spores sound improbable.

It grows slowly upon all ordinary nutrient media as a whitish to yellowish or brownish formation, and it grows very slowly upon gelatin. In the gelatin stab there are very fine points and teeth, and often branches are presented.

All gelatin plates present pinhead-shaped, prominent, yellowish-white, glistening, transparent colonies. Upon agar the growth possesses a slimy tenacity. Upon serum the organism thrives, although the growth is not much more abundant. The color is often quite a pronounced yellow.

In bouillon (at 37°) there is first turbidity; later a sediment; also a delicate, cobweb-like pellicle and a ring on the wall of the tube (optimum, 28°–32°).

The animal experiments are given so absolutely superficially that they are referred to with great difficulty. The organism (only one culture appears to have been employed) proved pathogenic for monkeys and swine. The three monkeys succumbed after a few weeks with a very complicated clinical picture, in which there appeared ulcerations at the point of inoculation, most extensive cutaneous eruption, nervous and cerebral disturbances, and indolent swelling of the glands.

At the autopsies there were found, in the first animal, ischemic alterations in the brain (areas of softening); in the second, leptomeningitis and endocarditis; in the third, hæmatocephalus internus. Of other disturbances there were, especially, multiple swellings of lymph-glands.

Microscopically, periarterial processes like those in syphilis appear, but the examinations are insufficiently penetrating throughout. The pathogenic organism was never successfully isolated again from one of the inoculated animals, and none of the animals was completely studied bacteriologically; in short, scarcely anything can be concluded from the animal experiments.

Van Niessen finally attaches value to the demonstration of an agglutination of his organism by serum from syphilitic patients.

Mycobacteria which Grow Luxuriantly at Room Temperature.

Literature.—The real discoverer of these organisms in butter is Petri. He pointed them out to R. Koch in July, 1896, who expressed his belief that they were different from true tubercle bacilli, which was also the opinion of Petri. A subsequently undertaken investigation by Lydia Rabinowitsch at Koch's suggestion appeared before Petri's work as a preliminary communication in the Deutsch. med. Wochenschr., Aug. 5, 1897, and in detail in Z. H. XXVI, 1897, 90. Petri's work was published (A. G. A. XIV, 1, 1898) after he had published a note concerning it in the H. R., Aug. 15, 1897.

Further important communications are: Hormann and Morgenroth (H. R., 1898, 217 and 1081); Moëller (Verh. d. Gesell. deut. Naturf. u. Aerzte, 1898; Deutsche med. Wochenschr., 1898; and C. B. XXV, 369); O. Schultze and Lubarsch (Z. H. XXXI, 153).

The most interesting discovery in the field of the morphology of bacteria during the past three years is to be considered that of the micro-organisms resembling the tubercle bacillus. Three years ago every acid-proof bacterium was a "tubercle or lepra bacillus"; to-day we know, through the harmonizing labors of many investigators[1] (Petri, Rabinowitsch, Moëller, and others) that acid-proof varieties occur not infrequently in the environs of men and domestic animals. Still, their differential diagnosis from the Mycobacterium tuberculosis appears quite easy, and yet the forms now known approach it in so many characteristics that, after the experiences with diphtheria and cholera, we must expect that still further difficulties will arise after more extensive studies. The exceedingly interesting forma piscicola of the Mycob. tuberculosis described on page 420 shows us to what a degree the true T. B. culture may change biologically (growth at room temperature, formation of a violet pigment in milk). Who knows how our newly discovered mycobacteria cultures may change if they are cultivated at incubator temperature or in the animal body for many generations?

To-day naturally it is not possible to consider the Myc. tuberculosis, without further knowledge, as a form of one of these organisms, which has been adapted to warm-blooded animals; but we may hope that the study of these latter will some time disclose to us much which now is dark in the matter of tuberculosis.

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[1] In manure acid-proof bacteria had already been found by staining: Severin (C. B. L. I, 98); Ferrán (C. B. XXII); Capaldi (Z. H. XXVI, 105).

In connection with Dr. Kumulis we have studied all the representatives of this group which we could obtain; in all, thirteen cultures. By detailed systematic comparison they arrange themselves into two varieties, of which one shows two or three forms.

Rabinowitsch has pronounced her organism (Myc. lacticola) identical with Myc. phlei. We find certain rather important differences, so that we believe for the present two species should be made. Lubarsch's observations, which we received after our own were entirely concluded, correspond in general very well with ours. The pathogenic properties are so similar that they may be treated together.

Mycobacterium lacticola *α* planus. L. and N.

(Plate 64.)

Synonyms. — Organisms resembling tubercle bacillus (Tuberculoseähnlicher) of Rubner-Obermüller, grass organism II of Moëller [1] (C. B. xxv, 369).

Microscopic Appearance. — Irregularly formed, shorter and longer rods of exceedingly variable length, straight or bent, partly acutely bent, often with clubbed swellings; generally in older cultures they are rather thick and often grown into threads of very irregular form. Branching is present (64, x; 63, VI, XII *a* and *b*).

They are not motile, and stain with methylene-blue, ordinary fuchsin, by Gram's method, and exactly like the true T. B. by the method for tubercle bacilli. [2] Growth occurs upon all nutrient media, taking place slowly at room temperature and somewhat more rapidly at incubator temperature, but always perceptibly faster than the T. B. Oxygen is indispensable. In agar shake cultures there is exceedingly little or no growth deep in the tube.

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[1] According to our observations, Moëller's grass organism II is the Myc. phlei.

[2] In smear preparations the staining property is lessened by agents employed in removing fat.

Gelatin Plate.—In young cultures the colonies are very similar to the Bact. coli macroscopically; later the colonies are more wrinkled. When magnified sixty times, they present a wavy, scalloped border with more or less wrinkling internally, being very similar to old colonies of the Bact. coli (64, VI, *a*). The deep colonies are not characteristic (64, VI, *b*).

Glycerin-agar Plate.—Macroscopically at first small, crumbly colonies, which later become wrinkled and elevated, and still later usually present a delicate transparent peripheral zone (64, IX). Magnified sixty times the colonies are usually pronouncedly granular, always becoming more transparent toward the periphery, and in this latter zone often present proteus-like markings (64, VIII).

Glycerin-agar Streak.—After six days at 37° a dirty grayish-white growth with a wavy smooth border, a fatty luster, numerous more or less elevated folds, and transparent in many places (64, II). Sometimes the wrinkling from the beginning is not so outspoken, the surface being much more homogeneous, and after many weeks it is of a yellow to a coppery red color. The consistency of the growth at first is like butter, later like saliva, and not crumbly and dry (64, III). Upon ordinary agar there later occurs a brownish-yellow coloration and little wrinkling (64, I).

Gelatin Streak Culture.—The growth was somewhat thinner and more delicate, the wrinkling more pronounced and regular. A decided orange color did not develop.

Bouillon Culture.—At first turbid, later clear. A pellicle is sometimes formed. The precipitate is moderate and yellowish-white. In sugar bouillon growth is more luxuriant, with almost constantly a thick wrinkled pellicle on the surface, the sediment being abundant and dissociated with difficulty. Milk is not coagulated, but after a time it becomes transparent and sometimes gelatinous. At the edge a bright orange pigment is deposited.

Potato Culture.—Slightly elevated, more or less wrinkled, often also a homogeneous growth, with notched or smooth border; when older, there are knobby elevations, the growth being bright to deep orange, with a fatty or moist luster (64, V).

Indol is formed in small amount and H_2S in both sugar bouillon and ordinary bouillon. Acid production is limited; in 10 c.c. of 2% grape-sugar bouillon as much as corresponds to 0.6 c.c. of decinormal sodium hydroxid. Gas is not formed in nutrient media containing sugar. Gelatin is not liquefied.

Distribution.—Found in milk, butter, grass; see page 435. For pathogenesis, see page 434.

We can separate the following from this only as a variety:

Mycobacterium lacticola β perrugosum. L. and N.

(Plate 63, I–VII.)

Synonym.—Butter organism of Rabinowitsch. We obtained two cultures which corresponded perfectly.

It is very similar to the Myc. lacticola *a* planum, and perhaps only a cultural form of the same, for Rabinowitsch described the young cultures as moist, thick, and creamy when cultivated direct from the animal body, and only after multiple passages through animals do they acquire the dry, early wrinkled character which this form presents.

Regarding the microscopic character, nothing special is to be said. We report, in detail, regarding the growths of these forms because of the interest which they now possess.

Glycerin-agar Plate.—Macroscopically in a short time there appear much wrinkled, irregularly dentate, dry scales, which later increase perceptibly in size. They are transparent, grayish-white, and are easily lifted from the surface and broken up (63, IV). When magnified sixty times, the smallest and youngest colonies are very granular; being fringed and torn at the periphery, grayish-white and transparent (63, III). Later they become opaque, brown to gray, with irregularly distributed markings, often spotted like a panther's skin (63, II). The gelatin plates are scarcely different.

Glycerin-agar Streak Culture.—Similar to the growth on the plates; there occurs here also an abundant, wrinkled, dry formation, which is extraordinarily like the growth of the true T. B. Especially when young, the cultures are easily mistaken for it. Later the growth becomes colored somewhat orange to coppery red (63, I) (Rabinowitsch).

In **bouillon cultures** a thick, wrinkled pellicle is formed very early. Still, the fluid remains clear, with scarcely any precipitate, indicating aerobic growth. There is a disagreeable ammoniacal odor (Rabinowitsch). In sugar bouillon the pellicle-formation is still more pronounced.

Milk Culture.—Like those of Mycob. lact. α planum.

Potato Culture.—At first there is a whitish to bright orange growth, with some elevation. After a short time it becomes wrinkled, and in older cultures this is especially prominent, similar to the appearance of glycerin-agar streak cultures. The growth is dull, rather dry, scarcely at all glistening. Indol is not formed; according to Rabinowitsch, there is a trace. H_2S is produced in ordinary bouillon only. Acid production: In 10 c.c. of sugar bouillon as much as corresponds to 0.8 c.c. of decinormal sodium hydroxid. There is no liberation of gas, and gelatin is not liquefied.

The **Bacillus friburgensis** described by Korn (C. B. XXV, 540) stands about midway between Myc. lacticola α planum and β perrugosum, and, according to our nomenclature, may be called **Mycobacterium lacticola** γ **friburgense** (Korn) L. and N. Upon glycerin-agar the streak growth at first is white, and more luxuriant than in planum; later there form sturdy, wrinkled elevations, which in time, especially at room temperature, become coppery red.

Korn gives the following as characteristic:

1. Stains by Ziehl-Neelsen's method especially well and is little influenced by acids.
2. Uniform growth—not interrupted—in gelatin stab.
3. The surface of the agar culture is depressed in the center, the peripheral zone being elevated.
4. Upon bouillon it produces a disagreeable but not ammoniacal odor. The characteristics are partly inconstant, partly unessential.

Inoculation with large quantities of pure culture and diseased organs causes no disease in guinea-pigs, rabbits, chickens, and pigeons. White mice are readily infected by the intraperitoneal injection of 0.5 c.c. of a suspension. The animals die in from four to forty days, and present massive nodules in all the abdominal organs.

Mycobacterium phlei.[1] **(Moëller.) L. and N.**

(Plate 63, VIII–XII.)

We here consider the micro-organisms described under the following names: "Moëller's manure organism," Moëller's grass organism I, from timothy grass, Petri's butter organism. Judging from his insufficient description, Petri appears to have often found also the Myc. lacticola, but he did not distinguish the forms. We do not believe any great value can be attached to the insignificant differences between these cultures (bouillon diffusely cloudy or only with a precipitate). The work of Dr. Kumulis will contain details regarding this. Obviously the timothy organism of Lubarsch also belongs here.

Literature.—Petri (A. G. A. XIV, 1). Lubarsch (Z. H. XXXI, 153).

Microscopic Appearance.—After growing for three or four days the rods are strikingly short and thick, and in this condition resemble the Corynebact. pseudophtheriticum very much. Later they become longer, sometimes clubbed; they also branch, and are then not distinguishable from the two preceding varieties (63, XII, *a* and *b*). They are not motile. The staining properties, intensity of growth, and relation to oxygen are just the same as in the Mycobact. lacticola.

Glycerin-agar Plate.—After a few days the macroscopic colonies are orange-red, with a wavy smooth border, without wrinkles and with a moist luster (63, XI). When magnified sixty times, the colonies are transparent, with a dark nucleus and markings like curls of hair. Toward the periphery is a delicate, transparent, more or less crumbly zone with a fringed, notched border (63, IX). Later the interior of the colony becomes darker and more opaque; only at the edge is there a delicate, veil-like zone.

Glycerin-agar Streak Culture. — Luxuriant, succulent, bright orange-red, homogeneous growth, which in time presents knobby elevations, but yet later, and especially in very old cultures, a considerable wrinkling appears, and then, except by the color, it is indistinguishable from Mycob. lacticola β perrugosum (63, VIII). In the gelatin streak culture the wrinkling is never so marked; in general, the growth on gelatin is somewhat less.

Bouillon Culture.—Sometimes there is a thin pellicle;

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[1] Timothy-grass is scientifically called *Phleum pratense.*

in other respects it is rather variable; the fluid is often almost clear, with a slight orange sediment, which rises up in columns upon shaking. At other times there is a slight transitory cloudiness. In sugar bouillon the growths are identical.

Milk culture exactly like Mycob. lacticola.

Potato Culture.—Like that of the glycerin-agar streak.

Indol is produced only as a trace, H_2S not at all, and also no gas is liberated. Acid production is the same as by Mycob. lacticola. Gelatin is not liquefied.

Pathogenic Effects of Mycobacterium lacticola and phlei.[1]

According to the descriptions of all authors, the pathogenic action of these varieties is so similar that it is not worth while to treat them separately.

It is ageed that the organisms are essentially less pathogenic without the simultaneous injection of butter, and even that the resulting peritonitis (adhesions, membranous formations, fibrinous and purulent exudates) may be dependent upon the injection of butter alone (Hormann and Morgenroth); still, the intraperitoneal injection of large quantities of pure cultures often produces an infection and causes the formation of nodules in the abdominal organs, yet the process often heals. If animals are killed three or four weeks after the injection of large quantities, the following conditions are found (Rabinowitsch): Slightly distended abdomen, more or less severe peritonitis, the peritoneum and mesentery beset with nodules, numerous small nodules beneath the intestinal serosa, mesenteric glands perceptibly swollen and often caseated. Likewise the liver, spleen, and kidneys show yellowish exudation in nodules in variable degrees. The lungs exhibit, at most, numerous transparent nodules, and are usually free from more serious disease. The transfer of small pieces of the organs to a new animal transfers the disease, according to Rabinowitsch; but according to Petri, and Hormann and Morgenroth, this only occurs if tuberculosis is simultaneously present.

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[1] Animal experiments appear to have been performed by Rabinowitsch, Hormann, and Morgenroth exclusively, and by Petri in part, with Myc. lacticola. Petri and Moëller have worked with Myc. phlei. Also Korn's experiments with his Myc. friburgense correspond except in unessential matters.

When butter is experimented with (4 to 5 c.c. of the previously thoroughly mixed mass, melted at 37° and containing butter-fat, watery part, and casein layers), if the bacilli are present in abundance, a fatal result often follows the injection after three to fifteen days. There are then found changes similar to those described above, only very much more intense, the abdominal organs being covered with well-developed inflammatory, fibrinous membranes which are swarming with the organisms.

Rabinowitsch found rabbits insusceptible in contrast to guinea-pigs. It is generally admitted that guinea-pigs are the most suitable experimental animals, although single reports concerning infection of rabbits are not lacking.

Korn could infect only mice with the pure culture of his Friburgensis (0.5 c.c. of a suspension or larger doses).

Whether injected into the blood-vessels, subdurally, or into the kidneys, the organisms produce more or less well-developed areas, such as described on page 416 in true tuberculosis: *i. e.*, formations resembling actinomycosis, but which disappear in the course of months. The animal body destroys the organisms which are introduced (Lubarsch and O. Schultze).

Also, in the structure of the accompanying miliary tubercles, these authors can often detect no difference from those of true tuberculosis.

Little has yet been learned concerning the distribution of the organisms resembling the Mycobac. tuberculosis. However, it appears very wide, for about 60% of butter samples in Berlin contained such organisms, and the investigations of Moëller demonstrated their frequent occurrence in manure, on grasses, etc. Lubarsch and Dieudonné verified this.

To obtain the acid-proof bacteria from butter, about 4 gm. are injected intraperitoneally in two guinea-pigs with a wide cannula, as indicated above. After about six to ten days the animals, if not dead, are killed, and cultures are prepared from the contents of the abdominal cavity which yield acid-proof bacteria, growing at room temperature.

To cultivate the organisms from grass, the grass is covered with water (Phleum pratense is especially recommended) and allowed to remain at 37° for thirteen to twenty-four hours. When frequent (every two hours) examinations have demonstrated that acid-proof organisms are present in quantity, plates are prepared.

It cannot be stated with certainty whether these new varieties of mycobacteria may also be pathogenic for man. So far as we know, no acid-proof organisms which grow upon all nutrient media and at room temperature have been cultivated from the sputum in cases of pulmonary diseases or from human organs.

Ginsberg stained numerous bacteria from two cases of eye disease, which were closely related to the T. B., but they were not cultivated (C. B. xxii, 62).

Flexner described a Streptothrix pseudotuberculosa Fl. from the lung of an old negro, growing with beautiful branching, staining by Gram's method, but imperfectly acid-proof with the T. B. stain, and not certainly pathogenic for guinea-pigs (Jour. of Exp. Med., iii, 435).

Differential Diagnosis of the Mycobact. tuberculosis (Tubercle Bacillus; T. B.).

1. If the T. B. is to be distinguished from bacteria which are not acid-proof, simple preparations are stained by the Ziehl-Neelsen method. Those which do not stain can be left out of question. T. B. which do not stain by the Ziehl-Neelsen method are unknown.

In the examination of sputum one proceeds as follows: The sputum is to be obtained as free as possible from foods and secretions from the mouth, and it is best collected in a sterile dish after the mouth has been well washed with water. Of the sputum, the more purulent (not mucous) portions, which are in lumps, are selected, spread upon the cover-glass, and stained (see Technical Appendix).

If no bacilli are found in a few preparations, although tuberculosis is suspected of being present, then one of the more searching methods described in the Technical Appendix must be employed in the attempt to discover isolated bacteria.

2. For the differentiation of T. B. from lepra and smegma bacilli the present methods scarcely suffice. T. B. are difficult to cultivate; lepra and smegma organisms are only very rarely cultivated successfully. For the tinctorial differences, see pages 422 and 425.

3. There is no difficulty in differentiating the T. B. from "pseudotubercle bacilli" which grow well at room temperature, so long as only one variety is present. Agar or gelatin plates are prepared and kept at 22°. True tubercle bacilli do not grow at all, at least when they come from warm-blooded animals, while the false varieties grow out well in two to four days. Reinoculations are made upon glycerin-agar and into bouillon in order to differentiate Myc. phlei and Myc. lacticola.

We are unable to suggest a method for recognizing culturally the true T. B. when associated with large numbers of bacilli resembling it; in cultures the true T. B. would be overgrown. According to the present state of our knowledge, a guinea-pig must be injected intraperitoneally with a moderate amount of the mixture.

If the guinea-pig dies after the intraperitoneal injection of small amounts of culture (one loopful) and without the addition of butter, with well-marked tuberculous changes in the abdominal cavity (enlargement of liver and spleen), and with involvement of the respiratory organs, these speak in favor of true tuberculosis. The histologic examination must give a predominance of true, giant-celled tubercles; and from the nodes and nodules organisms must be cultivated which will not grow at room temperature and on ordinary nutrient media, but at incubator temperature and upon ascites-glycerin-agar in the case of true T. B. Since death from tuberculosis in guinea-pigs usually occurs six weeks after the infection, before that the few germs resembling the T. B. are absorbed and have disappeared.

4. To determine whether a person or an animal is tuberculous, the injection of tuberculin is often made use of. Although it does not lack in individual contradictory results, yet there is no doubt that the tuberculin reaction constitutes a very important aid.

It is customary to inject cows subcutaneously with 0.3 to 0.5 c.c. of tuberculin, and to observe whether an elevation of temperature of 1.5° to 2° or 2.5° occurs after twelve to fifteen hours. A French commission speaks especially favorably of it (C. B. XIX, 645). The reaction sometimes is absent when the animal is extensively diseased, but for these cases the reaction is not required. It scarcely ever appears in healthy animals, and here it is to be remembered that small areas are often to be found with difficulty at the postmortem. The result is not influenced by other diseases in cattle. Exceedingly rarely, latent tuberculosis is stimulated to new activity. It is of importance that an animal often fails to give a positive reaction a second time for a month after a typical reaction was first obtained.

The question does not appear to have been investigated as to whether the tuberculin reaction occurs in animals infected with Mycob. lacticola and Mycob. phlei.

Naturally it is much more difficult to come to a conclusion regarding the reliability of the tuberculin reaction in man, since it cannot be controlled subsequently by thorough postmortem examinations; at any rate the injection of tuberculin in man for diagnostic purposes is not employed to any great extent.

3. Actinomyces. Harz, emend. Gasperini.

Growths upon solid nutrient media are elevated, tough, more or less wrinkled, often cartilaginous. Microscopically, they appear as long, thin, elongated mycelial threads, the young ones with homogeneous contents without partition walls, without a developed covering, and with abundant, true branches. In older threads there can be distinctly recognized a delicate membrane, and within it the colored contents, broken up into fragments. Positive cell divisions are rarely observed in cultures of varieties of actinomyces. Some varieties, in the animal body, present clubbed enlargements upon the ends of the radially arranged threads, which are to be explained as thickenings of the sheath (see below). Many species form chains of short colorless spores (conidia) upon thickened air hyphæ (two to ten times thicker than the threads) which rise above the solid medium and compact culture film like a white mold. Not infrequently chains of conidia are formed when the culture is submerged in river-water or 4% sugar solution ; yet here the spore chains are copiously branched, and the segmentation may even encroach far upon the threads (Gasperini, Lachner-Sandoval). Also some authors describe the occurrence of spore-like formations in the interior of the threads, but we have never certainly seen it. According to Lachner-Sandoval, this is only a fragmentation of the contents of the threads, which must not be interpreted as spore-formation even if it can be demonstrated that such fragments of threads cicatrize at the ends and later grow out again. They are not stained by the T. B. method, but always by Gram's method.[1]

Key to Some of the More Important Varieties of the Genus Actinomyces.

(*A*) *Pathogenic varieties*, with clubbed swellings of the ends of the threads in the animal body. Upon artificial nutrient media the formation of clubs is rare; conidia are sometimes produced in cultures, sometimes not.

(*a*) No growth below 22°, no growth on potato, no air mycelium, formation of clubs in artificial cultures very limited. Pathogenic for rabbits. **Actinomyces Hofmanni.** (Gruber.) Gasperini. Page 447.

(*b*) Grow below 22° and upon potato; formations of clubs in cultures scarcely ever observed.

1. Agar cultures, yellowish-orange, knobby, sometimes with air mycelium. Gelatin slowly liquefied. Typical club-formation in the body. Cause of the typical ray-fungus disease in cattle and man. **Actinomyces bovis.** Gasp. Page 440.

2. Agar growth, dry, granular, scanty. Pathogenic for cattle. Clubs have not been demonstrated in the animal. **Actinomyces farcinicus.** Gasp. Page 447.

3. Agar culture, a luxuriant, wrinkled, orange-yellow layer, with

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[1] For the limitations and naming of this genus, see Lachner-Sandoval, Ueber Strahlenpilze, Bonn, 1898; Sauvageau and Radais (A. P. VI, 242, Sur de genre Oospora); and our discussion on page 127. Regarding the species, the following articles are also important: Almquist (Z. H. VIII, 189, 1890), Gasperini (Annales de Micrographie, Bd. II, 449, 1890), and Annal. dell'Istit. d'Igiene di Roma, II, 1892, 166 (C. B. XV, 684). Rossi Doria (Annal dell'Ist. d'Ig. de Roma, Bd. I, 1892, 399). See also Berestnew (Z. H. XXIX, 94).

[2] While we ourselves naturally appreciate that this key is not satisfactory, our information does not allow us as yet to prepare a better one.

air hyphæ. Pathogenic for rabbits. Typical clubs formed in the animal. **Actinomyces asteroides.** Gasp. Page 449.

4. Agar growth whitish-red. Conidia are formed. Beautiful clubs in the animal. **Actinomyces maduræ.** Lachner. Page 452.

(*B*) *Non-pathogenic varieties:*

1. Growth colorless, nutrient medium brown. **Actinomyces chromogenes.** Gasp. Page 452.

2. Growth colorless, nutrient medium colorless. **Act. chromogenes.** Gasp. β alba L. and N. Page 455.

3. Growth colorless, nutrient medium colored violet. **Act. violaceus.** Gasp. Page 456.

4. For varieties with other colors, see Gasperini's **Act. carneus, albido-flavus, citreus,** etc., pages 451–456.

Actinomyces bovis. Harz.

(Plate 65.)

Synonyms.—Actinomyces bovis Harz, Act. bovis sulphureus Gasp., Nocardia Actinomyces de Toni e Trevisan, Streptothrix Actinomyces Rossi Doria, Oospora bovis Sauv. et Radais.

Common Names.—Ray fungi, Actinomyces.

Literature.—Israël (Virchow's Archiv, Bd. 74, 15; and 78, 421); Boström (Ziegler's Beiträge, Bd. IX, 1). "Actinomykosis" in Eulenburg's Realencyclopædie, Bd. I, 1894, by Birch-Hirschfeld. Grill (C. B. XVIII, 181).

Microscopic Appearance.—In the body of men and animals the organism forms sand-like masses, 0.2 to 0.6 or even as large as 1.2 mm. in diameter, of a gray, yellow, red, sometimes also green color, and when young, of a soft, and when older of a tougher consistency. The masses are made up of a ball of threads, the threads being radially arranged at the periphery and provided with characteristic, club-like formations, which are to be considered as derived from the gelatinous membranes of the threads (Boström). The threads terminate in the clubs, either free or with slight bud-like enlargements (Fig. 20, *a*, *b*). The threads show true branching, are thin (0.4–0.6 μ), partly without division, partly apparently composed of longer and shorter fragments. The surrounding "membrane" is very delicate. In the interior of the colonies, there are usually found between the threads, cocci-like formations, which originate from frequent fragmentation of the contents of the long threads, and later may be outside of the empty membranes (Fig. 20, *c*). These are not endospores! Older clubs become notched and cut, so that structures like an asparagus head may occur (Fig. 20, *a*). Often branched threads reach far beyond the zone with the clubs (Fig. 20, *d*). Sometimes clubs are entirely absent. Many actinomyces masses are dead when expelled in pus.

In cultures the branching mycelium is easily obtained (65, IX); the clubs are found only in the deepest layers of the nutrient medium.

Staining Properties.—The threads, but not the clubs, are best stained by Gram's method; afterward the clubs may be stained red with saffranin and diffusely staining carmine. According to Berestnew (Z. H. XXIX, 94), young actinomyces clubs stain by Ziehl's method, sometimes also by Gram's method.

Relation to Oxygen.—Grows aerobically and anaerobically, but better aerobically (Boström). The growth is limited.

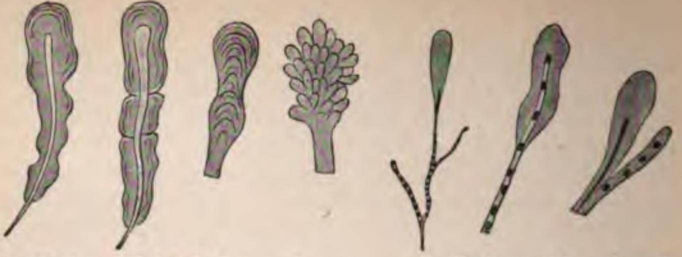
Chromogenesis.—The production of pigment is exceedingly variable; from white to various shades of yellow, orange, rusty, and brown appear to occur upon the various nutrient media; the darker tones at least predominate upon serum media, the brighter ones on gelatin.

Gelatin Plate.—(*a*) *Natural size:* After six days the colonies have a very irregular outline, are yellowish-gray, shining, sometimes fairly elevated above the surface of the gelatin, sometimes growing deeply into it (65, IV).

(*b*) *Magnified sixty times:* Dark yellowish-gray, homogeneously shaded colonies, sometimes presenting more or less distinct concentric rings. The peripheral zone is dark and beset with fine, curly hair (65, VII).

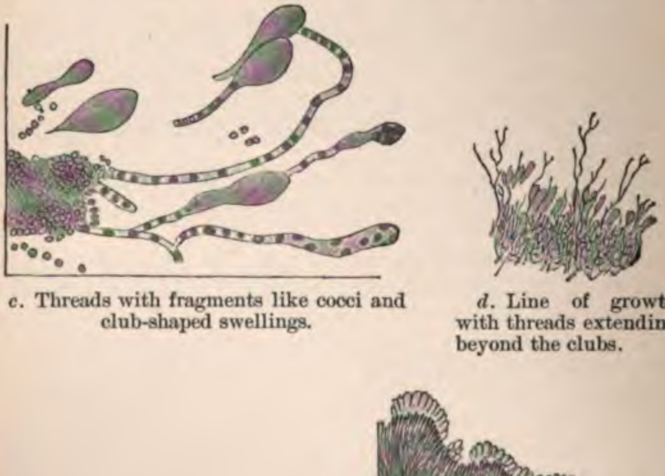
Gelatin Stab.—Surface growth at first is whitish-yellow, flatly elevated, faintly shining, rather tough; later the growth sinks into the gelatin with the limited liquefaction, leaving an air-space above. In the stab at first there are small yellowish-white clumps, which later have bristly outgrowths (65, III).

Agar Plate.—Macroscopically and microscopically



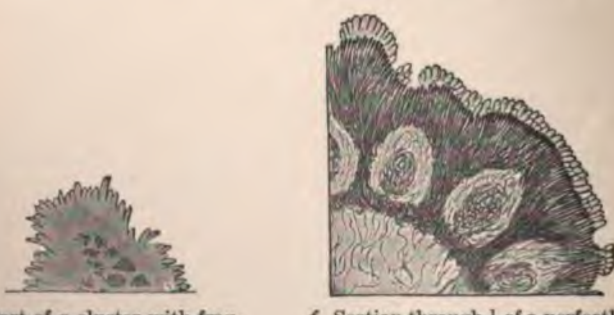
a. Various clubbed forms from fresh preparations.

b. Clubs with threads which contain fragments resembling cocci.



c. Threads with fragments like cocci and club-shaped swellings.

d. Line of growth with threads extending beyond the clubs.



e. Part of a cluster with fragmentation in the interior.

f. Section through ¼ of a perfectly developed cluster.

Fig. 20—Formation of clubs in Actinomyces bovis. Harz (after Boström). (*a*, *b*, and *c* are highly magnified—about 1000 to 2000 times; *d*, *e*, and *f*, slightly magnified.)

scarcely distinguishable from those in gelatin plates, except that the colors are fainter.

Agar Streak.—At first delicate, like dewdrops; then there slowly forms (after six to ten days) a whitish to whitish-yellow growth with an abruptly scalloped border, faintly lustrous and fairly elevated. This gradually comes to resemble a growth of Mycobacterium lacticola with its elevated paddings and ridges. After a very long time (thirty days) the growth gradually becomes dry, sinks in, and the color changes from white to yellow or brown. The culture appears to grow deeper into the nutrient medium, and often becomes surrounded by a more delicate zone, but in our cultures, in distinction to Boström's, no air hyphæ and no downy appearance was formed. The water of condensation remained clear.

Serum Streak Culture (after Boström).—At first the colonies are like dewdrops, which first become a little broader and thicker; then, extending out from some places, a whitish, velvety, dry covering is obtained. While the surface of the colony which is turned toward the serum gradually becomes colored from yellowish-orange to brick-red,—as do the older, puffed portions of the growth,—a delicate border of transparent bristling hairs is formed about the growth, in which later there form anew little buttons and puffs, which are first whitish and then change to yellowish or reddish.

Bouillon Culture.—The bouillon remains clear; at the bottom ball-like masses form, which are broken up with difficulty by shaking. Colonies upon the surface were never observed by us and rarely by Afanassiew. Microscopically the balls consist of threads with radially arranged fibers. Even in old bouillon cultures we could see no clubs.

Milk Culture.—Unchanged after eight days.

Potato Culture.—Slightly knobby, yellowish-white layer, closely attached to the potato, strictly limited to the streak. Often there occur distinct white, or yellow, and, according to Boström, also red spots (65, VIII).

Special Nutrient Media.—According to Boström, the fungus also grows in non-albuminous nutrient media, and even in sterilized water, as it does in bouillon. In an egg, Wolff and Israël obtained especially good dichotomous forms.

Conditions of "Spore-formation."—In many threads (not exclusively, but especially, with entrance of air) there are formed by continued transverse fission, short cocci-like and roundish oval bodies, which sometimes lie in close, but usually in broken rows within the empty and finally torn membrane (Boström, Kruse). Before the discharge of the "spores" the end of the thread is often somewhat swollen. The "spores" stain like protoplasm, not like the endospores of bacteria. We have seen nothing of these structures (Fig. 20, *c*) in either young or old cultures, although we took much pains to look for them. Typical conidia spores cut off in rows do not appear to be described.

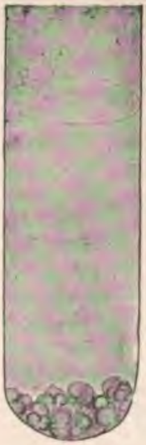


Fig. 21.—Bouillon culture of Actinomyces bovis.

Viability and Resisting Properties.—Very old cultures (nine months) are still alive.

Chemical Activities.—They have been but little studied. The odor is very faint, disagreeable but not moldy. From grape-sugar within eight days, neither gas nor acid is formed, and no H_2S in peptone bouillon.

Distribution.—

(*a*) *Outside the body:* They have not been found, but must frequently occur upon the beards of grains and grasses, because infection most often depends upon the penetration of a fungus-carrying barley barb, which is often found in the actinomycotic swelling (Boström). (Compare Berestnew, p. 447.)

(*b*) *In healthy body:* Never found.

(*c*) *In diseased human organism:* It is the cause of actinomycosis. Principal ports of entrance: (1) Mucous membrane of mouth and throat; (2) respiratory tract; (3) intestine; (4) skin. Almost always beards and other parts of grain are the vehicle; more rarely, wood. From the primary areas the fungus is carried to all parts of the body by means of wandering cells and emboli. The disease in man produces soft granulation tissue, which is not encapsulated, and has a tendency to break down and to spread slowly but extensively to the surrounding tissue (chronic phlegmon). The formation of fistulæ favors the extension. More rarely there are distinct tumors, as in cows. In the actinomycotic pus the actinomyces bodies are found. (See under Microscopic Findings.) There is scarcely a tissue or an organ of the body in which the actinomyces has not been demonstrated. Generalization of the actinomyces throughout the body is rare (see Messner, C. B. XIX, 487).

In 1892, 421 cases in man were known. Recently actinomycosis has also been observed in America.

(*d*) *In animals:* Especially in cows (rarely in swine, dogs, and horses). Formerly it was considered quite rare (1 in 10,000 to 1 in 3000), but it evidently is much more common, and simply overlooked. Sometimes it is epidemic. The localization is similar to that in man. Most often its seat is in the marrow of the upper and lower jaw; the marrow is traversed by soft granulation tissue and denser connective-tissue masses, the medullary cavity is enlarged, and new bone grows out from the periosteum (bony tumor). In other cases the soft parts of the face may be primarily attacked and the bones first involved from without. Also the pharynx and the wall of the stomach may be primarily attacked. The maxillary swellings were formerly described as white swelling, sarcoma of the jaw, spina ventosa, etc.; the disease of the tongue as "wooden tongue"; the growth in the lymph-glands as scrofula, etc.

Experimental Observations Regarding Pathogenic Action.—As opposed to many positive statements, Boström takes the stand emphatically that, in experiments on various animals, a multiplication of the introduced parasites is never observed, but only an encapsulation of the same. The most recent investigations of Wolff and Israël demonstrate that sometimes the short rods, when introduced intraperitoneally, grow out into threads and colonies. They did not succeed in producing a grave, progressive disease in the experimental rabbits, and after about seven weeks the organisms appear to die out. (Compare Mycobacterium lacticola and phlei, p. 434.)

Special Methods for Diagnosis and Cultivation.—*Diagnosis:* In man very often by recognizing the actinomyces clusters with the naked eye, or at least with the microscope (unstained or with a double stain).

Cultivation: For diagnostic purposes it is usually unnecessary. If a culture is to be made, it is best accomplished by means of numerous smears upon serum or ascites-agar, after the contents of the actinomycotic swelling have been thoroughly rubbed up in a mortar. The tubes are to be kept at incubator temperature and protected with rubber caps.

While Boström isolated the organism here described from all cases in men and cattle, Italian investigators especially claim that other closely related varieties may produce the clinical picture of actinomycosis; for example, the "**Actinomyces albus** Gasp." (See Gasperini, C. B. xv, 684, and p. 455.) The "**Cladothrix liquefaciens No. 2**" of Garten (C. B. xviii, 287) also stands very close. The **Actinomyces musculorum suis** Duncker (Zeitschr. f. Mikrosk. und Fleischbeschau III, No. 3), found in Berlin in the pale, watery muscles of quite a number of swine, is also related, but it has not been cultivated. There is the formation of clubs, but characteristic clusters are lacking.

The organism described by Kruse as the Strept. Israëli quite different.

Actinomyces Israëli. (Kruse.) L. and N.

Isolated twice from cases of human actinomycosis, grows best anaerobically, exhibits no branching upon ordinary nutrient media, and is readily inoculable into animals.

Other "**atypical or pseudo**" causes of actinomycosis have been described (in part, incompletely) by Berestnew (Z. H. xxix, 94, etc.). According to him, organisms resembling actinomyces can be very easily obtained if particles of barbs, etc., from grain are stuck into nutrient media which are subsequently kept in an incubator.

Actinomyces Hofmanni. (M. Gruber.) Gasperini.

Micromyces Hofmanni M. Gruber (A. H. xvi, 35).

This organism was obtained on one occasion from air in Vienna. It forms no air hyphæ, but the contents of the older threads of the fungus break up into cocci-like fragments. Especially beautiful is the formation of clubs exactly like those of the actinomyces and the observation of their final calcification (after several months) in bouillon cultures. It grows aerobically, and will only grow anaerobically if sugar is added to the medium. It does not grow below 22°, 37° being the optimum. Does not grow on potato and gelatin, and but poorly on serum and agar, but, on the contrary, it grows well upon most solid and liquid nutrient media with the addition of 0.5% to 3% of sugar. *Sugar-agar cultures:* Superficial colonies are elevated, sharply outlined, wrinkled, lusterless; the deep ones show a radiating structure with a delicate fringe. From sugar it forms acetic acid and some alcohol.

In animals, especially rabbits, it causes swellings filled with leukocytes and coagulated exudate, which then soften and heal by encapsulation, and which contain beautiful clusters.

Actinomyces farcinicus. Gasperini.

(Plate 66.)

Synonyms.—Bacille du farcin du Bœuf (Annales de l'Inst. Past., ii, 1888, p. 293). Nocardia farcinica Trevisan et de Toni.

Microscopic Appearance.—Short, segmented (knotty) threads with true branching. Nocard has photographed true branchings, but has interpreted them as false ones (66, x).

Spontaneous motion is lacking.

Staining Properties.—Stains with ordinary anilin dyes, and by Weigert's modification of Gram's method, in which anilin is used instead of alcohol after the iodin.

According to Nocard, it does not stain well with the common anilin dyes. According to Berestnew, it stains by Ziehl's method (Z. H. XXIX, 94); but according to Nocard, it does not. We could not repeat the tests, as our culture proved to be dead.

Requirements as Regards Temperature and Composition of Nutrient Media.—Not particular as to nutrient medium; grows at room temperature, and especially at 37°.

Gelatin Plate.—(*a*) *Natural size:* Scanty growth. After ten days there are only minute, round, transparent, shining colonies (66, V).

(*b*) *Magnified fifty times:* The superficial and deep colonies appear as smooth-edged, shining, gray to grayish-green masses in which no finer structure is distinguishable (60, VI).

Gelatin Stab.—Scanty; after twelve days the surface growth is whitish and granular; that in the stab crumbly (66, II).

Agar Plate.—(*a*) *Natural size:* The superficial colonies reach a size of 1 to 2 mm., are yellowish-white, irregular in form, and shining. The deep colonies remain tiny (66, VII).

(*b*) *Enlarged fifty times:* The surface colonies are similar to those on gelatin plates. The deep colonies are bright yellow, delicate, distinctly filamentous, and curly in structure (66, VIII).

Agar Stab.—About like the gelatin stab. Upon the surface of the agar there forms a whitish, coarsely granular mass, which is very irregular in shape because of fissures. The faintly colored growth shows air mycelium in spots (Nocard).

Agar Streak.—After eight days there is formed a gray to yellowish-white growth, consisting of transparent colonies, with a rough, finely fissured surface. The colonies are slightly or not at all connected. The water of condensation is clear, with slight grayish-white precipitate. After cultivating it for years we obtained growths which were actually more yellow and wrinkled, similar to our Act. bovis. The consistency became tough, while earlier it was crumbly.

Bouillon Culture.—Bouillon remains clear, with a moderate, slimy to tough precipitate, which is not entirely dissociated by vigorous shaking. Single colonies develop at the top as dirty gray pellicles with a dusty surface. Upon glycerin bouillon (according to Nocard) the pellicle is tougher.

Milk Culture.—Casein is dissolved without coagulation. Reaction alkaline.

Potato Culture.—Grows slowly (according to Nocard, rapidly), is whitish-yellow and lusterless. The surface appears as if beset with dry scales.

"Spores."—We have not observed them. Nocard described non-staining spores.

Distribution.—Cause of "Rinderwurms," "farcin du bœuf," occurring upon the island of Guadeloupe, and rarely in northern France. The clinical picture resembles that of cutaneous glanders, as also that of tuberculous affections of the cutaneous lymph-glands.

For animal experiments guinea-pigs are most suitable; then cattle and sheep. Rabbits, dogs, cats, horses, and the ass appear to be immune. In guinea-pigs intraperitoneal and intravenous injection is followed by death in nine to twenty days, with the clinical picture of miliary tuberculosis, yet the nodules contain a ball of the threads of the fungus (clubs?). Subcutaneous infection produces a very chronic disease in all susceptible animals, which answers to the picture of the spontaneous farcin du bœuf.

Actinomyces asteroides. (Eppinger.) Gasperini.

Synonyms.—Cladothrix asteroides Eppinger; Ziegler's Beiträge, IX, 287, good illustrations. Strept. Eppingeri Rossi-Doria.

Microscopic Appearance.—Rather sturdy, branching threads. When stained by Gram's method and faintly decolorized, as also in the fresh preparation, they have no distinct partition walls,[1] but many threads show a fragmentation into short, square ("cocci-like") segments, which (according to Eppinger), by opening of the thread membrane, may become free at the end (not observed by us). The branching, as represented by Eppinger, and as always seen by ourselves, is true branching, although he described it as false.

Spontaneous Motility.—The shorter threads exhibit a sluggish, and the shortest threads and spherical forms a very active motion (Eppinger). We have observed no motility.

Staining Properties.—Stain with all the anilin dyes and by Gram's method. According to Berestnew (Z. H. xxix, 94), they also stain by Ziehl's method.

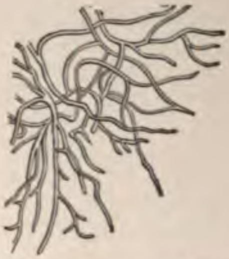


Fig. 22.—Oospora asteroides Sauv. and Rad. (after Eppinger).

Requirements as Regards Nutrient Media and Temperature.—Grows best at 37° and upon all ordinary nutrient media, but most luxuriantly on 2% grape-sugar agar. Upon gelatin at room temperature the growth is limited and the colonies are similar to those on agar. There is no liquefaction. Old gelatin cultures are distinguished by an orange-red color.

Agar Plate.—(*a*) *Natural size:* In the depth the colonies are round and slight. On the surface they grow well, and are circular, with a yellowish-white, faint, finely granular nucleus and a narrow, pale, peripheral zone.

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[1] By overstaining with hematoxylin and differentiating with glacial acetic acid-glycerin mixture, and counterstaining with eosin, Eppinger has convinced himself that the long threads consist of shorter and longer rods in a sheath.

(*b*) *Magnified fifty times:* When very young they are delicate, star-like, branching figures; later there gradually develops a denser, opaque center, with a delicate, ramifying peripheral zone.

Sugar-agar.—*Stab:* After twenty-four hours there is a small, whitish wart upon the surface, which gradually grows into a slightly elevated disk with a somewhat wrinkled surface and brownish-yellow color. The wrinkling, elevation, and extension of the growth increase for a long time; the periphery presents a delicate, flat, radially wrinkled border. In the stab there is only slight growth in the upper part. Upon ordinary agar the growth is more feeble and paler in color.

Bouillon Culture.—Delicate surface pellicle with white granules. The latter grow downward as tough masses (resembling drops of stearin), and then fall to the bottom, where a rich mass of the fungus gradually accumulates. The bouillon always remains perfectly clear.

Potato Culture.—At first a coarsely granular fillet, made up of snow-white nipples, and by degrees becoming wrinkled and brick-red. After about fourteen days a delicate, white, hairy covering develops at the periphery and gradually covers the entire red growth.

Spores.—Nothing is known regarding the resistance of the short segments known as "spores."

Distribution.—Found by Eppinger on only one occasion in the lymph-glands, and especially in a brain abscess and in the cerebral and spinal meninges of a glass-grinder, where it apparently was the cause of the disease.

Pathogenic Effects.—When introduced in various ways, it causes in animals (guinea-pigs, rabbits) a fatal disease resembling tuberculosis. (Pseudotuberculosis cladothrichica.) It has been shown by Lubarsch that it can form in animals colonies which are deceptively like actinomyces.

Actinomyces carneus. (Rossi Doria.) Gasperini.

Streptothrix carnea Rossi Doria. Closely related to the Act. asteroides. However, gelatin and agar cultures show distinct air mycelium, which gives the gelatin culture a pink, and the agar culture from a flesh to a reddish-orange color. It is not pathogenic for animals. The **actinomyces aurantiacus** (Rossi Doria) Gasperini is similar.

Actinomyces maduræ. (Vincent.) Lehm. and Neum.

Streptothrix maduræ Vincent (A. P., 1894, 129).

There is great similarity to actinomycosis in the course of the disease known as "madura-foot, madura-boil, Dehli-boil" (first brawny, then nodular, usually perforating externally). It is a disease, affecting especially the feet and hands, which is native to India, but also occurs in northern Africa, Italy, etc. Our description is from Vincent. In the pus from the fistulæ there are found bodies (gray, yellow, black) similar to those of actinomycosis, which, according to Kanthack's illustrations, have the same structure as actinomyces granules.

The organisms are obligate aerobes, and grow excellently upon decoctions of potato, turnip, etc., which have not been neutralized, with the production of alkali. As the best solid nutrient medium, Vincent employed a decoction of hay or potato to which is added, for every 100 gm. of gelatin, 4 gm. of glycerin and 4 gm. of grape-sugar. Gelatin is not liquefied. Old gelatin cultures resemble a vaccine pustule, being dry, closely attached to the nutrient medium, somewhat depressed in the middle, whitish, the periphery red. Upon potatoes whitish-red prominences, which often present an air mycelium with conidia; also in other mycelium, spore-formation occurs. There is no musty odor. The spores die in three minutes at 85° and in five minutes at 75°. The cultures with no spores die at 60° in from three to five minutes. The threads and conidia stain readily with all the anilin dyes and by Gram's method. It is not pathogenic for animals (rabbits, guinea-pigs, mice, cats).

Actinomyces chromogenes. Gasperini.

(Plate 67.)

Synonyms.[1] — Streptothrix chromogena Gasperini, Oospora Metschnikovi Sauvageau and Radais,[2] Streptothrix nigra Doria. Cladothrix dichotoma Macé, Günther non Cohn. Cladothrix odorifera Rullmann (C. B. XVII, 884, and C. B. L. II, 701). "Brauner Hesse."

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[1] We describe a variety isolated by ourselves; the synonyms we have determined in part from comparison with the descriptions, in part from the cultures.

[2] Sauvageau and Radias could not find spores in their Streptothrix schnikovii.

Microscopic Appearance.—True branching threads, often with perceptible separation into longer and shorter rods (67, x). There is no motility, but Rullmann has seen motion in the youngest stages.

On the air threads (see below), by continuous transverse division, short roundish members are formed (true conidia), which very readily fall off, and, upon germination, form new branching mycelium.

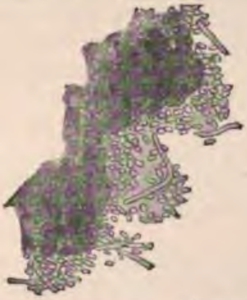

Fig. 23.—Actinomyces chromogenes. Gasperini. (Upper side of a bouillon pellicle magnified about 700 times.)

Staining Properties.—With all anilin dyes and by Gram's method.

Relation to Oxygen.—Grows better aerobically.

Requirements as Regards Temperature and Nutrient Media.—Thrives upon all ordinary nutrient media at room and incubator temperature, more rapidly at the latter.

Gelatin Plate.—(*a*) *Natural size:* At first the colonies are brownish, round, slightly elevated, tough, dull, and begin first in the center, rarely at the periphery, to take on a whitish, chalky condition. Hereupon are formed concentric, wide, white rings; the drier (thinner) the nutrient medium, the more rapidly there occurs a more or less complete overgrowth of the colony, with white air hyphæ, and therewith a chalky appearance. The gelatin near the colony is colored dark brown and is slowly liquefied, so that finally round, pea-sized, chalky crusts float in shallow cups (67, v and vi).

(*b*) *Magnified sixty times:* Very young colonies consist of a confused ball of threads; older ones appear but slightly transparent, with zones having wavy, jagged boundaries, all of which are darker in their peripheral portions. The edge of the colony is beset with delicate threads, like a fringe, which extend outward into the discolored gelatin (67, vii).

Gelatin Stab.—The surface growth is like that on the gelatin plate. Sometimes drops of fluid (no oil!) are seen upon the surface of the growth. The gelatin is very slowly liquefied from above downward. In the stab the short, radiating tufts of fibrils, which even at first develop, can be observed for a long time (67, i).

Agar Plate.—(*a*) *Natural size:* As upon gelatin.

(*b*) *Magnified sixty times:* After about six days no structure is distinguishable in the dense colonies; they are dark and homogeneous and surrounded by distinct fringes (67, viii).

Agar Stab.—Upon the surface the growth at first is rather moist, with a yellowish luster and elevated like the head of a nail; later it is drier, tough, and somewhat puffed. The agar is discolored brown. In the stab there are radiating, bristle-shaped branches (67, iii and iv).

Agar Streak.—The growth spreads out only moderately, presents (after four to six days) a brownish color, and on the thinner parts of the agar a white, chalky, peripheral zone. In the course of time the whole becomes whitish and chalky. Upon the clear water of condensation there later forms a tough, brownish film, which also develops chalky, white air hyphæ, especially on the glass walls (67, ii). At other times a clumpy growth is present at the bottom of the water of condensation without any pellicle.

Bouillon Culture.—At first a delicate and later a dense pellicle. In grape-sugar bouillon there are thick, clumpy, radially arranged masses on the bottom. The bouillon becomes brown.

Milk Culture.—Dense, yellowish-brown to cinnamon-colored layer on top. The milk is clarified and alkaline.

Potato Growth.—Growth quite rapid and luxuriant. As early as forty-eight hours in the incubator, a yellow, yellowish-brown, greenish-brown, or brown layer, 8 mm. wide, has formed. In our cultures the chalky appearing air hyphæ always began at the edge. The potato is later discolored intensely brown to black, and becomes strongly alkaline.

Chemical Activities.—There is produced a dark-brown pigment and an intense moldy odor upon all nutrient media. According to Rullmann, the earthy smell is most intense upon bread pap and other media composed of carbohydrates. There occurs a nitrogenous body, soluble in water and ether. The odor is said to be the same as arises from an unclean floor upon washing. Ammonia is abundantly formed. According to Rullmann, in symbiosis with fission-fungi it has considerable capacity for forming nitrate.

Distribution.—(*a*) *Outside the body:* In Würzburg it is not uncommon in the air, soil, and water, and appears also otherwise distributed.

(*b*) *In organism:* We found it once in gastric contents.

Special Methods for Demonstration and Cultivation.—Agar plates in incubator. Observe brown halo, chalky discoloration, odor.

Actinomyces chromogenes Gasperini β alba. L. and N.

Streptothrix Foersteri Gasperini (Cohn?), Streptothrix alba Rossi Doria, Streptothrix I and II Almquist, Oospora Guignardi Sauvageau and Radais, Actinomyces albus Gasperini,[1] Oospora Doriæ Sauvageau and Radais.

According to Doria, it is especially frequent in Rome, but occurs also in Würzburg. It does not color the nutrient media. It forms a white cushion of circular form, and tends to produce abundant air-spores. Gelatin is liquefied.

According to Gasperini, the cultures many times exhibit a sudden production of pigment, like the Actin. chromogenes. According to Rossi Doria, it also grows upon fucus (sea-weed) nutrient media with a dark discoloration of the nutrient medium. From what we have seen and learned from the literature, the only possible comprehension of this form appears to be as a variety of Act. chromogenes. According to Gasperini, this form may cause actinomycosis in cattle.

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[1] The **Cladothrix invulnerabilis** Acosta y Grande Rossi (C. B. XIV, 14) appears closely related. It is said to bear heating for one-quarter hour to 120°. The cultures have an earthy odor.

Actinomyces violaceus. (Rossi Doria.) Gasperini.

Found many times in Rome by Doria. It liquefies gelatin. The nutrient media are discolored. Gelatin becomes bright wine-red, agar grayish-violet, potato reddish-brown.

Streptothrix aurantiaca, citrea, albido-flava have been described by Rossi Doria (*l. c.*), and their standing as species must be proved by further comparison. According to Gasperini, they are all to be considered as actinomyces.

Lachner-Sandoval (*l. c.*) has studied in detail the **Actinomyces albido-flavus** Gasp. He describes the germination of the conidia as accompanied by elongation, many times following two directions simultaneously. The vegetative forms were killed by 70° in three minutes; the conidia by 80° in five minutes.

A "streptothrix" which is not acid-proof, and which possesses pathogenic properties for animals, was isolated by Rullmann from sputum (Münch. med. Wochenschr., 1898, 919). Upon Löffler's serum it furnished chrome-yellow growths, otherwise they were colorless. Often the cultures give off a moldy odor.

Actinomyces erysipeloidis. (L. and N.) Lachner-Sandoval.

Synonyms.—Oospora erysipeloidis L. and N., Streptothrix Rosenbachii Kruse.

As the cause of the rare sporadic disease, chronic erysipelas, erythema migrans, "erysipeloid" of Rosenbach, the latter author has described a true branching microorganism, related to the "cladothrix," but often occurring in the form of short rods and spheres. The threads often terminate in "a thick point." The description of the cultures reminds one most of mouse septicemia. In all growths they become brownish. It grows best at about 20°, less well at incubator temperature. When inoculated into man, it causes non-febrile, intensely itching, sharply outlined redness, which spreads slowly.

Actinomyces necrophorus.[1] (Flügge.) L. and N.

Bacillus of diphtheria in calves (Mitteil. des Kais. Ges. Amts., II). Bac. diphtheriæ vitulorum Flügge and B.

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[1] Since Flügge gave both the above names simultaneously, we are free to choose between them. The species designation of "necrophorus" seems to us to be especially happy.

necrophorus Flügge, Necrosis bacillus Bang (C. B. XIII, 201). Streptothrix cuniculi Schmorl. Actinomyces cuniculi Gasp. (Deut. Zeitschr. f. Tiermed., XVII). The organism lies in the necrotic tissue (diphtheritic membrane, etc.) on the side turned away from the surface as long, often radially arranged meshes or bundles, separated from healthy tissue by a narrow, necrotic zone. It is an obligate anaerobe, growing best on blood-serum or blood-serum agar at incubator temperature, and has been incompletely described as to its morphology (branching!). It is of great practical interest as the cause of numerous diseases in animals. It was cultivated by Schmorl from a destructive epidemic in rabbits, and first described by Löffler as the cause of calf diphtheria (mouth, larynx, nose). According to Bang, it also causes in young and old cattle, horses, and swine the most various necrotic affections (panaritium, gangrenous pock, intestinal diphtheria, liver abscess, vaginal and uterine diphtheria, etc.). We were unable to study this evidently important organism.

Regarding thermophilic varieties of actinomyces, see Kedzior (C. B. L. III, 154) and Tsiklinsky (C. B. XXV, 385).

After subcutaneous inoculation of mice, Löffler obtained the picture of progressive connective-tissue necrosis. A layer of lardaceous infiltration extends subcutaneously from the point of infection and envelops the kidneys, liver, and intestine with yellowish masses of exudate. According to Löffler, rabbits are not affected characteristically; but according to Schmorl and Bang, they are. All investigators found other experimental animals to be immune.

APPENDIX II.

Higher Fission-fungi. (Higher Fission-algæ.)[1]

The close relationship with the chlorophyllaceous algæ is still more evident in the varieties of this section than in all the forms previously discussed, and many investigators consider them as true algæ. On the other side, the connection with the simple fission-fungi is still so close that at least a brief mention seems necessary.

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[1] We have had little personal experience with this group, and limit ourselves partially to a critical review of the literature.

In distinction to the true bacteria, the members of this group have this in common, that the threads can be recognized as having a basal (not growing, often attached) and an apical (growing, free) end.[1] The ends are often of different thickness.

Key to the Recognition of Some of the More Important Genera of Fission-algæ.

Threads without distinct sheaths:

(*a*) Without sulphur granules. **Leptothrix Kützing**, see below.

(*b*) With sulphur granules, motile, not attached. **Beggiatoa Trevisan**, page 461.

Threads with sheaths:

(*a*) Without sulphur granules.

1. Without pseudodichotomous branching. **Crenothrix Cohn**, page 463.

2. With pseudodichotomous branching. **Cladothrix Cohn**, page 465.

(*b*) With sulphur granules. **Thiothrix Winogradsky.**

Leptothrix epidermidis Biz.[2]

(Plate 69.)

Microscopic Appearance.—In our preparations there are sturdy unbranched threads, distinctly jointed and readily falling apart, with no evident distinction as to base and apex. Young cultures show bacilli about like the B. mesentericus (69, XI and XII).

Motility.—The young rods present distinct motion like that of bacilli. We were unable to stain flagella.

Staining Properties.—Stains with all anilin dyes and by Gram's method. With iodin and iodid of potassium alone there is no blue stain.

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[1] We must certainly admit that the discernment of the two ends has often caused greater trouble than was to be expected from the statements of Bücher, for example, in the case of Beggiatoa.

[2] Before the leptothrix of the mouth had been cultivated we could place the L. epidermidis among the "true" leptothrices only with reservation. (See p. 461.)

Relation to Oxygen.—Grows better with the admission of oxygen.

Requirements as to Temperature and Nutrient Media.—Grows luxuriantly and rapidly at room and incubator temperature upon all nutrient media.

Gelatin Plate.—(*a*) *Natural size:* At first, minute white points which liquefy the gelatin as soon as they become a little larger—after twenty-four to forty-eight hours. The older colonies exhibit a small white flake in the center of the liquefied areas; also the edges of the areas present a whitish border (69, VIII).

(*b*) *Magnified fifty times:* Young, superficial colonies present a pretty convolution of curly, wavy threads. The center soon becomes dark and cloudy and sinks in, while there remains a row of fine radiating hairs as a peripheral zone. As the liquefaction advances there is finally a flat, gray saucer, which presents a delicate hairy border toward the solid gelatin, and in the center of which is a curly mass whose structure becomes more and more indistinctly crumbly (69, IX).

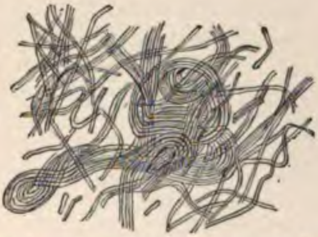

Fig. 24.—Leptothrix epidermidis Biz.

Gelatin Stab.—After twenty-four hours there is formed a conical area of liquefaction with whitish flocculi, and at the apex crumbly, yellowish-white masses of bacteria gradually accumulate. After four or five days there forms a grayish, tough pellicle on the surface (69, I).

Agar Plate.—(*a*) *Natural size. Superficial colonies:* White or yellowish white, sharply outlined growths with smooth but irregularly notched borders. The *deep colonies* remain small, dense, yellowish-white (69, V).

(*b*) *Magnified fifty times. Superficial colonies:* The center is opaque, brownish-yellow, passing gradually into a yellowish-gray peripheral zone which consists of very closely set, fine, radiating hairs (69, VI). *Deep colonies:* Roundish, nodular, yellowish-brown, here and there presenting single or bunched projecting hairs (69, VII).

Agar Stab.—*Surface growth:* Yellowish-gray to brown, rather smooth-bordered, slimy. Gradually it becomes dull, with some whitish elevations. In the stab the growth is like a grayish-white thread. After several months the agar becomes colored dark brown (69, III and IV).

Agar Streak.—Like the surface growth of the stab culture. Upon the water of condensation is a tough, wrinkled, brownish pellicle. Where this passes on to the tube-wall it is pure white. The water of condensation is clear (69, II).

Bouillon Culture.—Only on the surface there is a tough, thick, wrinkled pellicle, which is firmly adherent to the glass.

Milk Culture.—On the surface a dense scum. The milk is not coagulated, and while the milk becomes transparent, a limited white precipitate is formed.

Potato Culture.—There is rapidly formed an elevated, reddish to grayish-brown, sharply outlined growth. In time the surface develops wavy wrinkles. In old cultures the peripheral zone presents a white, chalky discoloration (69, X).

Spores.—There are no endospores.

Distribution.—Found by Bizozzero upon the skin of healthy men.

Related Varieties.—This variety resembles in many points the subtilis-mesentericus group. Wavy growth, vigorous liquefaction, and air hyphæ upon potato are observed, in which respects its cultures are very similar to those of the B. subtilis. The variety is more properly called Bacillus epidermidis. To be sure, it must then be taken for granted that accidentally a spore-free form of bacillus is exemplified in this variety.

Regarding the forms designated as "**Leptothrix buccalis,**" which occur frequently in the mouth, especially in deposits on the teeth, little can be said that is satisfactory, since cultures have almost always failed.

Miller (Die Bakterien der Mundhöhle, II. Aufl., Berlin, 1894) appears to have cultivated no leptothrix. He cites the following uncultivated leprothrices, briefly and very insufficiently characterized:

Leptothrix gigantea Miller. Threads fixed at one end, small to very thick, with or without distinct septa. Iodin reaction?

Leptothrix maxima buccalis Miller. Jointed threads 1 to 1.3 μ thick, without iodin reaction.

Bacillus maximus buccalis Miller. Like the preceding, but with iodin reaction. It is not stated why this species is designated bacillus while the former are designated leptothrix.

Leptothrix innominata Miller is said to present slender, 0.5 to 0.8 μ thick, tangled, unsegmented, often wavy or bent threads, which sometimes stain violet with iodin.

Arustamow (C. B. VI, 349) described two unnamed leptothrices of the mouth, cultivated by him, both of which grow at incubator temperature, No. 1 being an exquisite anaerobe, No. 2 an outspoken aerobe. The agar growth corresponds in some measure to that of the L. epidermidis; potato and gelatin growths are not described.

Dobrzyniecki (C. B. XXI, 225) has described in detail a **Leptothrix placoides alba,** which was successfully cultivated aerobically on gelatin. The growth at first resembled anthrax, and then liquefied. The growth on agar was slow—hard, dense colonies being formed. The organism presents long, jointed threads, with a tendency to form tangles, and it stains blue with iodin and iodid of potassium solution and a little lactic acid. Stains by Gram's method. Is not motile.

Flexner has isolated an interesting organism from a rabbit which died of puerperal infection. It occurs in long threads, is always free of spores, has no motion, and does not branch. It is pathogenic, but very difficult to cultivate outside of the body. He names it **Bacillus (Leptothrix?) pyogenes filiformis** Flexner (Jour. of Exp. Med., Vol. I, 211, 1896).

Beggiatoa alba. Vauch.

Long and rather thick (1 to 5 μ) unbranched threads, without membranes, and quiet or with a gliding motion. When fresh, there are in part no partitions, but often numerous very highly refracting bodies may be recognized. The granules consist of sulphur, which may especially be recognized if the threads are previously allowed to dry, and also by their solubility in H_2S. In this way also the previously indistinguishable transverse partitions become apparent. According to Winogradsky, the breaking up of the threads into smaller pieces, which later grow out, is the only method of multiplication. The statements of Zopf regarding other forms in the cycle of development of Beggiatoa are opposed by Winogradsky.

According to the old idea, Beggiatoa formed H_2S and sulphur from sulphates, and was the cause of H_2S being present in sulphur springs. According to Winogradsky, on the contrary, it is dependent for its nourishment upon the preexisting H_2S, which it transforms into sulphur. (See Untersuchungen über Schwefelbakterien, C. B. II, 590.)

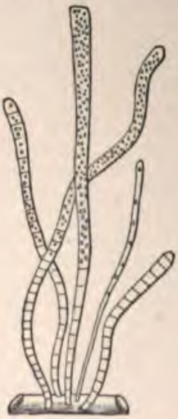

Fig. 25.—Beggiatoa alba Vauch (after Zopf).

B. alba is found especially in foul slime and dirty water; also sometimes as isolated individuals in pure water. If abundant, they form whitish films.

B. nivea Rabenhorst is recognized in sulphur springs as the principal constituent of the slime in the spring.

B. roseo-persicina. Zopf. (Die Spaltpilze, 3. Aufl.)

Bacterium photometricum Engelmann (Pflüg. Arch., Bd. 30, 95).

This variety is very striking because of its rose-color. In the cooler parts of the year it spreads widely along the banks of small streams, pools, etc. It is always a sign of contamination of water, but not specific (perhaps from sulphite wood-pulp factories or the like). According to Winogradsky, Zopf has improperly included in this variety a large number of other rose-colored inhabitants of water.

Jegunow (C. B. II, 279) has reported many interesting observations regarding another sulphur bacterium, whose classification is still undetermined, but it most likely belongs to the spirilla.

Crenothrix polyspora. Ferd. Cohn.

(Cohn's Beiträge, Bd. I, H. II, 130.)

Long, rigid, unbranched threads, consisting of a single row of low cells, unpigmented, included by a membrane which is very thin at the younger parts of the thread and thick at the older parts. The membrane is a product of the cuticle of the cell. In the membrane is deposited some iron hydroxid or carbonate, which stains it brown. Sometimes also the membranes for considerable lengths are surrounded by a yellow, ferruginous mass with a luster like oil, so that macroscopic, brownish flakes appear.

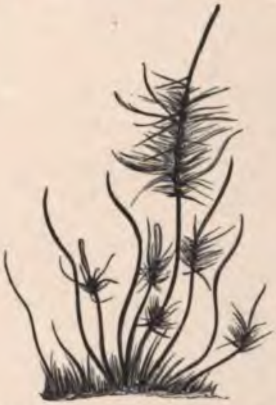

Fig. 26.—Crenothrix polyspora. Cohn.

The thickness of the threads varies from 1.5 to 5.2 μ, it often being easily recognized that the older part of the thread (where it is fixed) is wider and stronger. Also the height of the individual cells varies from one-half to four times the thickness, square forms being most common (Fig. 27, *a* and *e*).

Sometimes the terminal cell of a thread is large and oval (like a spore), in which case a deeper cell grows out laterally.

Propagation occurs through a peculiar breaking up of the cells at the end of a thread into fragments. Cohn distinguishes two types: (*a*) Formation of microgonidia: several individual cells in the thread break up by longitudinal and transverse division into not less than 16 very small plasma spheres, which later are freed from the somewhat swollen ends of the threads and grow out into new threads (Fig. 27, *d*). (*b*) Formation of macrogonidia: several of the cells in a thread near its end are transformed by infrequent division into larger, roundish, oval or diplococci-like forms, which grow out into new threads (Fig. 27, *b*). One type passes over into the other.

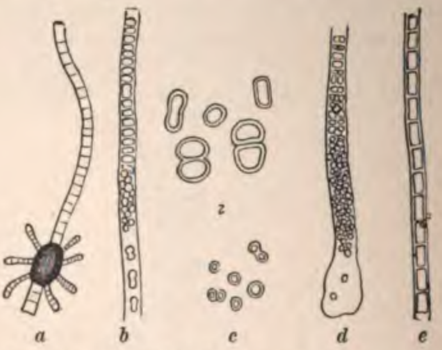

Fig. 27.—Crenothrix polyspora. Cohn.

The plant is widely distributed (especially) in waters containing iron (also in tap-water). A pure culture in the bacteriologic sense has not been obtained. According to Rössler, cultivation readily succeeds in well-water in which pieces of brick are boiled and to which is added "some" us sulphate. (Compare Ferd. Cohn, Beiträge zur

Biologie, Band I, Heft I, p. 108, Breslau; Rössler, Arch. f. Pharm., Bd. 233, 1895.)

Here also should be included the interesting **Leptothrix ochracea** Kützing, which does not belong to the leptothrices in a strict sense, because of its fully developed membrane. Winogradsky, who describes it in detail, gives the following characteristics: slender, jointed, fixed threads of bacilli, with membranes. The membrane is thick below, thin at the free ends, and the terminal rods are entirely without any membrane. The bacilli in the membrane are motile, but this motility is lost as soon as the membrane has reached a certain thickness. This variety thrives only in water containing ferrous oxid. In the metabolic process hydrated oxid of iron is deposited in the membrane. In hay decoction prepared from well-water and freshly precipitated hydroxid of iron the organism always grows easily and rapidly. It forms yellowish flakes and films, and in nature gives rise to extensive ocher deposits. (Compare Winogradsky, Bot. Zeitung, 1888, p. 261.)

Cladothrix dichotoma. Ferd. Cohn.

(Cohn's Beiträge, Bd. I, Heft. III, p. 185.)

Long, apparently non-segmented threads, with thick or thin membranes, in part free, in part attached to putrefying algæ. Thickness 1 to 5 μ. The pseudodichotomy is especially interesting. It is dependent upon the growing of a lower segment of a thread along by the side of a higher one (Fig. 30). Pure cultures of this organism have been but little studied; we have not possessed any. According to Büsgen, the most recent investigator of this organism (Ber. der deutsch. bot. Gesellschaft, 1894, p. 147), it grows slowly and without perceptible liquefaction in gelatin containing a little meat extract.

The surface growth consists of a "round white patch," which is not elevated, and from which, as from the stab, after a few days delicate threads grow out.

The threads have thin membranes when grown on gelatin and thick ones in dilute solutions of meat extract. The membrane is patent at the end of the threads, and through this opening, as also through irregularly occurring tears of the membrane, there pass (by means of unilateral bunches of flagella, 8 to 12 μ long, according to G. Fischer, see Fig.

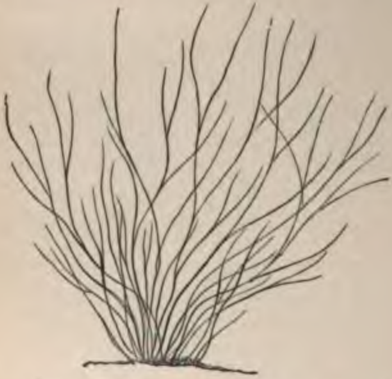

Fig. 28.—Characteristic appearance of Cladothrix dichotoma Cohn (after Migula).

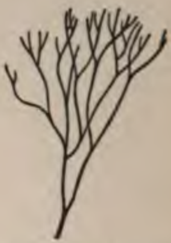

Fig. 29.—Characteristic appearance of Cladothrix dichotoma Cohn (after Cohn).

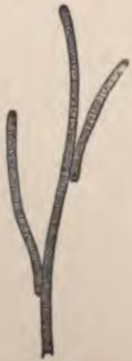

Fig. 30.—Pseudodichotomous branching of Cladothrix dichotoma Cohn (after Cohn, reduced).

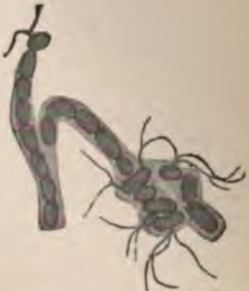

Fig. 31.—Separation of flagellated organisms of Cladothrix dichotoma Cohn (after A. Fischer).

31), short, actively motile bacilli, which, after wandering, become fixed by one end and again form new threads.

There are no "spores" nor "sporangia" unless the occasionally occurring expansions of the threads in which the rods lie in double rows are so called.

APPENDIX III.

Notes Concerning Insufficiently Elucidated Diseases Which Perhaps Depend upon Bacteria.

Of the diseases not yet mentioned in this book the following are outside of our consideration, because:

(*a*) Dependent upon higher molds: favus, herpes tonsurans, the deep wound suppurations caused by hyphomycetes, certain mold mycoses.

(*b*) Caused by yeast fungi: many tumors in man and animals.

(*c*) Dependent upon protozoa: malaria, dysentery (?), Texas fever in cattle, Surra or Tsetse disease, variola.

Some diseases which are probably produced by fission-fungi, as syphilis, are treated briefly in the text; some others are suited only to a discussion in an appendix, because that which is known regarding their etiology is either very uncertain or so incomplete that the insertion of the micro-organisms in a system is not possible.

Phlyctenular (Scrofulous, Eczematous) Ophthalmitis.

In contrast to the authors who would recognize in the Micrococcus pyogenes alone the cause of the above-mentioned inflammations of the eye developing upon a scrofulous substratum, a number of investigators have shown that in carefully selected uncomplicated cases, in a majority of the examinations, no micro-organisms were to be found in the conjunctival phlyctenulæ and recent corneal infiltrations. See Axenfeld (C. B. XXIV, 194).

Beri-beri.

Regarding this important tropical disease, the literature contains the most heterogeneous statements. The cause of the disease is recognized by Musso and Morelli (Compt. rend. de la Soc. de Biolog., 1893, 18) in an organism which is very closely related to the Micr. pyogenes α aureus; by Hunter, in agreement with Pekelharing and Winkler, in a white, motile staphylococcus (C. B. XXIV, 537).

Besides, there are those who conceive of beri-beri as a chronic intoxication (from sea animals), and place it in the same class with pellagra, etc. See Grimm (C. B. XXIV, 538).

Articular Rheumatism.

While some authors believe articular rheumatism is due to the Micrococcus pyogenes (see p. 184), Bannatyne, Wohlmann, and Blaxall (C. B. XX, 400) believe they have found its cause in a short, fine bacillus (2 μ long, 0.6 μ thick) which usually stains at the ends (eighteen positive cases!). In bouillon it slowly grows as small, fine puncta and crumbly particles. From the bouillon scanty growths upon agar and Löffler's serum may be obtained. According to the English writers, the organism is constantly found in the joint fluid; more rarely, and only in severe cases, in the blood. Leyden has found in five cases of endocarditis (C. B. XIX, 722) a fine diplococcus which can hardly be cultivated at all, and which may possibly be the cause of the disease.

Hospital Gangrene.

Vincent (A. P., 1896, 488) observed in Arabs, who were infected in Madagascar, in the typical cutaneous ulcers, very abundant, straight, rarely slightly bent, non-sporulating bacteria which do not stain by Gram's method. In sections it is easily demonstrated in the characteristic location. The organisms lie below the characteristic pseudomembrane in abundance. For their demonstration the organs are first hardened in concentrated sublimate solution, then in alcohols of increasing strengths. The sections are stained ten minutes in cold phenolthionin solution, placed in alcoholic solution of iodin a few seconds (0.01 iodin in 200 alcohol), washed with alcohol, and finally counterstained with safranin. The inoculation of animals was successful only when streptococci, B. coli, pyocyaneum, etc., were also inoculated with the special bacterium. The organism appears strikingly similar to those found in stomatitis ulcerosa (see below). Also sometimes abundant spirochætæ are found in hospital gangrene.

Pneumonia in Cattle.

(Péripneumonie des bovidées.)

It does not lie within the scope of this book to speak of the cause of this disease in detail, while, on account of its extreme minuteness, nothing definite can be said of its form even when magnified two thousand times. The cultivation was primarily successful when small, thin, collodion sacs, containing bouillon and a trace of the fluid from the lung of a sick animal, were placed in the abdominal cavities of living guinea-pigs. After fifteen to twenty days the sacs were removed, and the fluid was found very slightly cloudy because of the above-mentioned, most minute, motile objects. By means of the organisms which have been transplanted repeatedly upon artificial nutrient media, cattle may be infected in the characteristic manner, and the organism again be cultivated in vitro in peptone solution to which a few drops of serum have been added. (See Nocard and Roux, Annales de l'Inst. Pasteur, 1898, 240.)

Measles.

Canon and Pielicke (C. B. xiv, 287) claim to have found constantly in fourteen cases of measles a bacterium which is most variable in size (very minute to 3.4 μ), and which stains interruptedly with a mixture of 80 c.c. of saturated aqueous solution of methylene-blue and 20 c.c. of 0.25%

eosin solution (in 70% alcohol) after three hours at incubator temperature. Only from the sixth day of the disease on, are the organisms found in preparations, which does not bespeak an etiologic significance for them. Still, the discoverers consider this organism, which does not stain by Gram's method, and cannot be cultivated (only upon blood-bouillon many times a slight growth appears), to be the cause of measles.

Recently Czajkowski (C. B. XVIII, 517) has found similar organisms in the blood, which he has portrayed and cultivated upon glycerin-agar, but especially upon blood-glycerin-agar. The growth is delicate, scanty, and like dewdrops. The organism is pathogenic for mice. It is motile and is not stained by Gram's method.

Mouth and Foot Disease.

In opposition to numerous defective investigations which have recognized the cause of this disease in most variable sized and sometimes easily cultivated fission-fungi (compare Stutzer and Hartleb, A. H., 1897, 372), Löffler and Frosch have determined that the cause of the foot and mouth disease is indeed present in the contents of the mouth and foot vesicles, but that it is so minute that it passes through dense bacterial filters and is invisible with the best microscopes. Only after repeated filtration through the densest filters does the lymph lose its infectious properties (C. B. XXIII, 371).

Myxoma Disease.

The cause is so far unrecognizable. The disease occurred spontaneously in Sanarelli's rabbits in Montevideo. It manifests itself in conjunctival catarrh, leonine swelling of mouth and nose, inflammatory swelling of the urinary and genital organs and buttocks, especially in hyperplastic changes at the places where the skin and mucous membranes join. Also, myxomatous or gelatinous subcutaneous swellings which are very vascular are found in various parts of the body. In most animals dying from the subacute disease the postmortem reveals hypertrophy of the lymph glands, orchitis, and swelling of the spleen. The disease is easily produced in rabbits by inoculation with the perfectly clear and apparently entirely sterile serum from other cases. Also dogs and men react to the inoculation (C. B. XXIII, 865).

Noma.

Petruschky has found diphtheria bacteria together with pseudodiphtheria bacteria in two cases of noma faciei, which he cured with diphtheria antitoxin (Deut. med. Wochenschr., 1898, 600).

Epidemic Parotitis.

Laveran (Compt. rend. de la soc. de Biolog., 1893, 95) found diplococci in the blood and organs in 67 out of 92 cases of mumps. They kill mice and cause in rabbits and dogs a transitory orchitis, which often is associated with mumps also. Mecray and Walsh (C. B. XXI, 68) have made similar observations. The cultures were described by the latter authors as very similar to those of very poorly growing Microc. pyogenes.

Diseases of Plants.

In spite of the efforts of numerous investigators to demonstrate bacteria as the cause of diseases of plants, we possess little knowledge which is free from objection in this field. The botanist Alfred Fischer takes an especially skeptical stand regarding the statements so far made (C. B. L. v, 279), while Erwin Smith, who has himself done much work in this field, is much more optimistic in his judgment, and considers the connection of bacteria and disease to have been demonstrated in a whole series of instances. He enumerates some of the varieties of bacteria which are most certainly pathogenic for plants (C. B. L. v, 271; in the same place the literature is given). We refer to the following: **Bacillus amylovorus** Burrill (cause of a disease of apple and pear trees), **Bacillus oleæ** Savastano (tuberculosis of the olive tree), **Bacillus hyacinthi-septicus** Heinz, **Bacillus tracheiphilus** Erw. Smith (injurious to various cucurbitaceæ), **Bacillus solanacearum**

Erw. Smith (injurious to cruciferæ). Besides, there have been described bacterial diseases of vines, celery, and sugar-beets, yet only exceptionally are the descriptions sufficiently complete.

Rachitis.

Mircoli ascribes it to Microc. and Strept. pyogenes (C. B. xx, 321).

Acute Rheumatism.

It may be mentioned that Sawtschenko, with Achalme, has recently recognized the cause of acute rheumatism in an anaerobic, sporulating bacillus. Confirmation is awaited with interest (C. B. XXIV, 794).

Cattle Plague.

We only know that the causative agent does not pass through a clay filter: the German investigators in South Africa consider the statements, accompanied by illustrations, of Nencki, Sieber, and Wyznikiewicz (C. B. XXIII, 529) as delusions. They recognize the cause of the plague in a small, spherical organism, but do not directly designate it a micrococcus. The organism is said to grow in peptone solution.

According to Abbe, the limit of the working capacity of our microscopes lies at 0.1 to 0.2 μ.

Scarlatina.

Many of the older writers especially, and of the newer ones,—for example, d'Espine (C. B. XVIII, 132),—are of the opinion that the specific cause of scarlatina resides in the streptococcus which is very frequently present, but this is almost certainly not true. Czajkowski (C. B. XVIII, 116) never failed to find in the blood of seventeen cases of scarlatina a diplococcus (according to the illustrations, more like short diplobacilli) which grows to a limited extent upon solid nutrient media (glycerin-agar, blood-agar, serum), more luxuriantly in fluid nutrient media, and is pathogenic for mice. It is not stated whether the diplococcus appears as a streptococcus upon fluid nutrient media. The great tenacity of the cultures is striking.

Doehle (C. B. XII, 906) and L. Pfeiffer consider protozoa to be the cause of scarlatina.

Ulcerative Stomatitis and Angina.

Bernheim (C. B. XXIII, 177) described two organisms as frequently or constantly present in the ulcers of the gums and tonsils:

1. A bacillus resembling the B. diphtheriæ (once noticed to be motile), somewhat larger than it, with pointed ends, often more or less curved, staining rather faintly with Löffler's methylene-blue, and decolorized by Gram's method if the alcohol acts a long time.
2. A fine spirochæte, which does not stain by Gram's method, similar to the spirochætæ of the teeth.

Neither could be cultivated.

Similar results have been obtained by many other writers, as Vincent and Abel. (See Abel, C. B. XXIV, 1). The results obtained by Vincent in hospital gangrene in Madagascar are strikingly similar; also both organisms were present. (Compare p. 468.)

J. Seitz has described as the Bacillus hastilis a widely distributed (tonsils, etc.), long, slender organism with pointed ends, which has not been obtained in pure culture. It appears to be closely related to Bernheim's organism of stomatitis ulcerosa. From non-saccharine bouillon it forms foul-smelling gas (Z. H. XXX, 47).

Trichorrhexis Nodosa.

According to Marcusfeld, caused by a sporulating bacillus, perhaps from the subtilis group. The relation to the disease is uncertain (C. B. XXI, 230).

Typhus Exanthematicus.

Lewaschoff (C. B. XII, 635, 728; XVIII, p. 132) claims to have cultivated a characteristic Micrococcus exanthematicus in pure culture upon ascites-agar from the juice of the spleen or blood from the finger in 118 cases of typhus fever. It is strikingly motile, always in the blood, and grows anaerobically. Both in the blood and in cultures many, but not all, of the individuals present one or two very long, motile, spiral appendages, which take the stain for flagella. Lewaschoff calls this form of remarkable spirochæte, exanthematica. According to his opinion, there is perfect agreement between his findings and the newer investigations of Ljubimoff (cocci), Calmette and Thoinot (A. P., 1892, 39) (egg-shaped bodies and spirilla), von Dubief and Bruhl (C. B. XIV, 17), Curtis, and Combemale (diplococci).

APPENDIX IV.

Essentials of Bacteriologic Technic.

The following directions and short explanations include about all the technical material which is given in a thorough bacteriologic course. We have introduced only those things which are essential and, according to our experience, practical, without referring to the literature. More details will be found in the books mentioned in the preface.

I. Microscopic Examination of Bacteria.

1. Hints upon Microscopic Technic.

For bacteriologic examination we use almost exclusively the modern microscope with Abbé's illuminating apparatus, iris diaphragm, and a low-power and an oil-immersion objective.

(A) **Low magnification (60 to 100 times)** with a narrow diaphragm is used in the minute examination of plate cultures. In this examination either the cover[1] is removed and the colony examined from above, or, if one does not wish to contaminate the plate by exposing it, the dish is laid upon the cover and the colony examined from below, but this does not give such characteristic pictures in all cases.

(B) **High Magnification. Oil-immersion Objective (700 to 1200 times).**—This finds its use in the examination of single individuals. A drop of cedar oil is placed upon the preparation (slide, cover-glass) and the tube is lowered by means of the coarse adjustment until the lens just touches the oil; then it is accurately adjusted upon the preparation with the micrometer screw.

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[1] Our plate cultures are always poured into dishes.

(a) **Unstained preparations.** Narrow diaphragm! They are examined in two ways:

1. A drop of pure culture in a fluid medium or a little drop of water with a trace of pure culture mixed in it is placed between the slide and cover-glass; or, better,

2. *In a hanging drop.* A drop of a pure culture in a fluid medium or a drop of bouillon in which is mixed a minute quantity of a pure culture is placed upon a cover-glass; the cover-glass is then turned over and placed upon a hollow ground slide so that the drop is suspended within the hollow. The cover-glass is now fixed to the slide by applying a very little water to each corner of the cover, or, if the observation is to be more prolonged, by means of vaselin.

(b) **Stained preparations.** Open diaphragm! Abbé's illuminating apparatus. In the examination of sections with a double stain, the wide diaphragm is required for the bacteria, the narrow opening for the tissues.

(C) **Cleaning the Preparations and the Microscope.**—The immersion oil is always gently brushed off, and now and then quickly cleaned with xylol and chamois skin; the setting of the lens is loosened by prolonged action of the xylol. Also immersion oil dried upon the cover-glasses of old preparations is readily removed by xylol.

2. The most Important Solutions for Use in Making Preparations.

(A) Staining Solutions.

1. **Aqueous Alcoholic Solutions of Fuchsin and Methylene-blue.**—A concentrated "stock-solution" is prepared by pouring absolute alcohol upon the pulverized dyes (fuchsin, methylene-blue) in bottles, and after shaking and allowing them to stand a few hours, they are filtered. Of this saturated solution 1 part is mixed with 4 parts of distilled water, and before using is filtered. In order to obtain good preparations it is better to stain a longer time with weaker solutions than for a short time with strong solutions.

2. **Carbol-fuchsin (Ziehl's Solution).**—

Fuchsin	1.0 gm.
Acid. carbolic. liq.	5.0 "
Alcohol.	10.0 "
Aq. dest.	90.0 "

3. **Anilin Fuchsin.**—Four parts of anilin oil (anilin. pur.) are well shaken several minutes with 100 parts of distilled water, and then filtered until all the water has passed through clear (then the funnel is removed, since otherwise oil will also pass through). In this anilin water 4.0 gm. fuchsin are dissolved and it is again filtered.

4. **Anilin Gentian-violet (Ehrlich's Solution).**—To 100 c.c. of anilin water, 11 c.c. of a concentrated alcoholic solution of gentian-violet (stock solution) is added. This solution does not keep long.

Czaplewski and E. Fränkel recommend that instead of anilin water, 2.5% carbolic acid water be employed. This has the advantage that the solution does not decompose so soon as anilin mixtures.

5. **Löffler's Methylene-blue.**—To 100 c.c. of water is added 1 c.c. of 1% solution of potassium hydroxid, and 30 c.c. of concentrated alcoholic solution of methylene-blue. The staining property of the dye is intensified by the addition of the alkali.

6. **Acetic acid methylene-blue,** according to M. Neisser, for the staining of granules:

(*a*) 1.0 gm. of methylene-blue is dissolved in 20 c.c. of 90% alcohol, and to this is added 950 c.c. of distilled water and 50 c.c. of glacial acetic acid.

(*b*) 2.0 gm. of Bismarck brown are dissolved in 1 liter of boiling distilled water (filter!).

7. **Bismarck Brown.**—Prepared like No. 1 (stains tissues, but stains bacteria poorly).

8. **Alum Carmin.**—In 100 c.c. of a 5% solution of alum 2 gm. of carmine are placed. It is boiled for an hour and filtered. It stains nuclei and tissues, but not bacteria.

9. **Eosin.**—For staining the tissues about the bacteria. Two gm. of eosin are dissolved in 100 c.c. of distilled water.

10. **Safranin.**—Three gm. of safranin are dissolved in 100 c.c. of hot distilled water.

(B) Differentiating Agents.

1. **Distilled water.**
2. **Absolute alcohol.**
3. **Iodin, iodid of potassium solution** (Gram's solution):

Iodin. pur.	1.0 gm.
Potassii iodid.	2.0 "
Distilled water	300.0 "

4. **Sulphuric acid,** 25%.
5. **Acetic acid,** 3%.
6. **Acid alcohol:**

Alcohol (90%)	100 c.c.
Distilled water	200 "
Pure hydrochloric acid	20 drops.

(C) Mordants for Staining Flagella.

1. **Löffler's mordant:** 10 c.c alcoholic solution of fuchsin; 50 c.c. cold saturated solution of ferrosulphate; 100 c.c. 20% solution of tannic acid.

2. **Bunge's mordant:** 25 c.c. of officinal solution of ferric chlorid, diluted twenty times; 75 c.c. of a saturated aqueous solution of tannic acid. Just before using, enough of a 3% solution of hydrogen peroxid dded to give a reddish-brown color, and it is then filtered. (We always dispensed with this last addition.)

(D) Clearing and Mounting Agents.

1. **Xylol.**
2. **Canada balsam.**
3. **Dammar varnish.**

3. Preparations of Stained Specimens of Bacteria.

(A) Smear Preparations.

1. Simple Stain with Fuchsin or Methylene-blue.—This may be used for all bacteria except the tubercle bacillus.

A drop of distilled water is placed upon a cover-glass (cover-glass preparation) or upon a slide (slide preparation), and in it is mixed a trace of pure culture (preferably from a solid nutrient medium), and the drop is then spread out in a very thin layer. After the fluid has evaporated, the preparation is passed quickly through the flame three times with the film up in order to fix the bacteria to the glass (avoid burning!). The film of bacteria is then covered with the staining solution. After a short time (a few seconds to a minute), perhaps with gentle heat, the preparation is washed with water and allowed to dry (perhaps again with gentle heat). Finally the dry cover-glass is fixed upon a slide by means of a drop of Canada balsam, the bacterial side being down.

It may here be remarked that slides and cover-glasses upon which the drop of water does not adhere or does not spread out evenly are best cleaned with soap. Alcohol and ether, which are much used for cleaning purposes, do not remove the particles of fat so completely.

2. Gram's Stain.—

1. The smear preparation is made as above.
2. Stain with Ehrlich's solution three minutes.
3. Wash with water.
4. Differentiate with Gram's iodin solution one minute.
5. Decolorize with absolute alcohol until no more color is removed (usually one or two minutes).

If the alcohol does not decolorize rapidly enough, it can be accelerated by placing a drop of anilin-xylol upon the preparation and then again washing with alcohol.

6. Dry and mount.

According to our experience, the current idea that every variety of bacterium either stains well or not at all when treated by this method is false. Thus, we observed, in the case of the Bact. fluorescens, for example, which is usually said in the literature not to stain, that three out of twelve different cultures, of twenty-four hours' growth, stained very beautifully. According to Zimmermann, all the varieties of Bact. fluorescens stain in young cultures.

In like manner we found the bacillus of symptomatic anthrax from a culture to stain, although it has often been said not to stain. The contradictory results may be easily explained in part as due to varying age or youth of materials experimented with, and to variations in the differentiating process with alcohol. The Bacillus tenuis, which has been designated above as failing to stain by Gram's method, upon a later test with the same culture and technic stained very well. In every case whenever the test is to be made, a fresh anthrax preparation should be simultaneously stained, and all preparations should be subjected to the action of alcohol for the same length of time (one or two minutes). Then we can judge very well whether a variety of bacterium retains or gives up the stain. A separation of the individual genera and varieties by Gram's stain now seems scarcely at all possible, since there are found within a single genus all stages, from those organisms which stain well to those that stain poorly.

The following may usually be stained by Gram's method:

Micrococci (not the **Micr. gonorrhœæ**).

Sporulating bacilli (**symptomatic anthrax** and **malignant edema** are uncertain).

Of the **non-sporulating bacilli: Proteus, mouse septicemia, swine erysipelas, lactic acid bacterium.** (Uncertain: **fluorescens,** some bacteria not unlike the coli Bact.)

Genus **Corynebacterium, Mycobacterium,** and **Actinomyces.**

The following usually do not stain:

Micr. gonorrhœæ, Bact. influenzæ, Bact. coli and **typhi, Friedländer's Bacillus pneumoniæ, bacteria of pest** and **glanders,** the **vibrios** and **spirilla.**

3. Demonstration of Capsules.—The following is Johne's method: (1) Cover the preparation with 2% solution of gentian-violet and warm until it steams; (2) wash in water; (3) moisten with 2% acetic acid for six to ten seconds; (4) wash with water.

By this method also in varieties which are not regarded as "capsule-bacteria," a distinct membrane may often be demonstrated about the intensely stained bacterial cell. The capsule is seen most beautifully when examined in water.

4. Staining of Flagella.—The flagella, which are almost always invisible when unstained, are usually demonstrated according to Löffler's method:

1. Making the preparation (rubbing up a trace of young agar streak culture—not bouillon culture—in a very small drop of water, spreading well, and drying rapidly).
2. Heating the preparation with the mordant (p. 476) until it steams (not boils!) for one-half to one minute.
3. Wash with a vigorous stream of water.
4. Wash in alcohol to remove the remains of the mordant adhering at the edge.
5. Cover with staining solution (a few crystals are dissolved in 10 c.c. of anilin water, and to this is added, drop by drop, 0.1% sodium hydroxid solution until the clear fluid just begins to become opaque—precipitation) and warm until it steams for one minute.
6. Wash with water, dry, and mount in Canada balsam.

Extreme cleanliness is essential in the manipulations, especially very thorough cleaning of the cover-glasses with acid and alcohol, or, still better, with soap. Also the cultures must be young, although it is not essential, as some authors claim, to employ cultures which are only twenty-four hours old. We have often obtained very good preparations even after twelve days. We usually use freshly prepared mordant.

According to Löffler, the addition of a very definite amount of acid or alkali to the mordant is required for most kinds of bacteria in order to obtain well-stained flagella. Löffler recommends that to 16 c.c. of the mordant there be added:

Cholera vibrios	½– 1 drop	1% NaHO.
Spirillum rubrum	9 drops	" "
Bacterium typhi	20–22 "	" "
Bacillus subtilis	28–38 "	" "
Bacillus œdematis maligni	36–37 "	" "
Bacterium pyocyaneum	5– 6 "	equivalent H_2SO_4.

Our results show that, in the majority of cases, very satisfactory pictures are obtained with the original mordant, and that the addition of alkali or acid is by no means essential. Similar results have been obtained by other writers; for example, Lucksch, Günther, A. Fischer, Nicolle, and Morax.

Recently Bunge has employed a somewhat different method, which has given us good results also, but, like Löffler's process, it has also sometimes left us capriciously in the lurch: (1) The preparation is made as for Löffler's; (2) warming with Bunge's mordant (p. 476) until it steams for one minute; (3) careful washing with water and drying; (4) staining with slightly warm carbol-gentian-violet or carbol-fuchsin; (5) washing with water, drying, mounting in Canada balsam.

The silver method of van Ermengem is also much employed for staining flagella. The cover-glass preparations are stained with a mixture of 1 part 2% osmic acid and 2 parts 10% to 25% solution of tannic acid, to 100 c.c. of which 4 to 5 drops of glacial acetic acid are added. It is then washed with water, and afterward with absolute alcohol, and the preparation is next moistened for a few seconds with 0.5% to 2.5% silver solution. The preparation, without washing, is brought into a solution of tannic acid 3.0, gallic acid 5.0, sodium acetate 10.0, and water 350, for a few moments, and again back to the silver solution until it begins to get black. Finally it is again washed in water.

Most of our preparations have been made with Bunge's mordant when it was several months old.

5. **Staining of Endospores.**[1]—According to Hauser:

1. Preparation of the film. (It is recommended to pass it through the flame ten times, instead of three.)

2. Stain with watery fuchsin or carbol-fuchsin (Ziehl's solution), the preparation being covered with abundance of the staining solution and warmed above a flame for one or two minutes until it begins to simmer (not boil). The fluid which evaporates is constantly replaced by fresh staining solution.

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[1] Arthrospores possess no undisputed tinctorial reaction. Regarding metachromatic bodies, Ernst's and Bunge's granules, preliminary stages of spores and their demonstration, see page 21.

3. Wash with acid alcohol[1] (p. 476) until the preparation no longer appears red.

4. Counter-stain with methylene-blue (a few seconds).

The spores remain red, the bacilli appear blue.

6. Staining of Tubercle Bacilli.—This is accomplished under the same principles as spore staining. The preparation is treated with a hot, actively staining solution, and afterward with some acid solution. Everything except the tubercle bacilli is decolorized.

(*a*) The preparation is stained (**according to Ziehl-Neelsen**) exactly the same as spores, except that it is passed only three times through the flame. We employ this method exclusively.

Another favorite method is the one recommended by A. Fränkel and Gabbet, in which decolorization and counter-staining are accomplished at the same time. Then the preparation, after being stained with hot carbol-fuchsin and washed with water, is placed in the following solution: Sulphuric acid, 1; distilled water, 3; pulverized methylene-blue, enough to produce a most intense blue color. It is then again carefully washed in water, dried, and mounted in Canada balsam.

Although this method is convenient, it is better for those without much experience to carry out the staining, differentiation with acid, and counter-staining separately, since in this way success is more certain.

(*b*) **Ehrlich-Koch's method** is also often employed. The preparation, after being dried and fixed in the flame, is treated with hot anilin gentian-violet solution for one to two minutes, and then with acid (usually 30% nitric) for one to four seconds, and for a few seconds with 60% alcohol. It is then placed in an aqueous solution of Bismarck brown for a few minutes and washed in water. The tubercle bacilli now appear violet upon a brown background.

These methods are suitable for cover-glass preparations made from pure cultures and from tuberculous sputum containing many tubercle bacilli. If very few or no tubercle bacilli are found in the first preparations, then some method must be employed for concentrating them. We give two of the innumerable ones which are recommended:

(*a*) *According to Strohschein:* 5 to 10 c.c. of sputum are mixed with three times the quantity of Wendriner's borax-boracic acid solution,[2] and, after vigorous shaking, the mixture is set aside for four or five days to settle. The mixture becomes fluid and the bacilli settle to the bottom. Such sputum may be used for examination after years.

(*b*) *According to Dahmen, modified by Heim:* The entire sputum is boiled in a steam chamber for fifteen to twenty minutes and allowed to cool. The opalescent fluid is poured off and the crumbly sediment used for smear preparations.

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[1] In place of acid alcohol, 30% nitric acid or 5% to 25% sulphuric acid may be employed, but they must be allowed to act for a shorter time.

[2] Eight gm. of borax are dissolved in hot water, 12 gm. of boracic acid are added, and then again 4 gm. of borax; after crystallization it is filtered.

7. Neisser's Stain for Diphtheria Granules.—The requirements for good success with the stain, according to Neisser, are as follows:

The preparation is stained one to three seconds with acetic acid methylene-blue, rinsed off, counter-stained two to five seconds with Bismarck brown.

1. The cultures must be grown upon Löffler's serum, solidified at 100°.

2. The cultures should not be under nine nor over twenty to twenty-four hours old.

3. The cultures must be kept in an incubator at 34°-35°, not above 36°.

8. Stain for the Organisms of Syphilis.—According to Lustgarten the preparations are stained for twenty-four hours in anilin gentian-violet, placed a few seconds in 10% permanganate of potassium solution, and quickly decolorized in dilute sulphuric acid. The syphilis organisms are dark violet to steel blue; the tissues are decolorized.

(B) Preparation of Sections.

1. Universal method according to Löffler, adapted to almost all bacteria:

The sections are carried upon a German silver or glass spatula from alcohol to Löffler's alkaline methylene-blue solution, where they remain for five to thirty minutes. They are then placed for a few seconds in 1% acetic acid, and after differentiation are passed through alcohol and xylol and mounted in Canada balsam. It must be determined how long the acetic acid may be allowed to act, and the dehydration in alcohol must not be prolonged any more than is essential. The bacteria should be blackish-blue, the nuclei blue, the protoplasm bluish.

2. Nicolle states that by the following method he has obtained satisfactory staining in sections in the case of bacteria which stain with difficulty; for example, glanders, typhoid, etc.:

Löffler's blue one to three minutes; wash in water; 10% solution of tannic acid a few seconds; wash in water; absolute alcohol, oil of cloves, xylol, Canada balsam.

3. Gram's method: (1) Ehrlich's solution, three minutes; (2) Gram's solution of iodin, two minutes; (3) alcohol, one-half minute; (4) alcohol containing 3% hydrochloric acid, ten seconds; (5) alcohol several minutes until maximum decolorization; (6) xylol, mount in Canada balsam.

If it is desirable to have a contrast stain of the tissue, after maximum decolorization in alcohol, the sections are placed in an aqueous solution of Bismarck brown (10 : 100) for a few minutes, then again for fifteen to twenty seconds in absolute alcohol, then in xylol, and finally mounted in Canada balsam.

4. Botkin maintains that Gram's stain is facilitated by washing preparations which are stained with anilin gentian-violet in anilin water. The preparations, when removed from the iodin solution, subsequently bear the action of alcohol much better. In this way it is possible to stain the Bac. œdematis maligni and Bacterium pneumoniæ Friedländer.

5. **Gram-Weigert method** (stain for fibrin, also good for bacteria): The sections are stained with anilin or carbol gentian-violet, washed in 0.6% sodium chlorid solution, and dried upon the slide with filter-paper. The iodin solution is then applied and the excess again removed with filter-paper. The section is then decolorized with anilin oil until no more color is given up, placed in xylol, and mounted in Canada balsam.

6. **Kutscher's modification of Gram's method**: There is prepared a concentrated solution of gentian-violet in a mixture of anilin water 1 part, alcohol 1 part, 5% carbolic acid water 1 part. Of this concentrated solution a drop at a time is added to a watch-glassful of water until a shimmering layer forms upon the surface. In this the sections are placed, and after ten to fifteen minutes are washed in water, then placed in the iodin solution for a minute, then in alcohol, xylol, and mounted in balsam. By this method malignant edema and symptomatic anthrax are also stained.

7. If **tubercle bacilli** are to be stained in sections, carbol-fuchsin or anilin gentian-violet are used as for cover-glass preparations, except that the heating is omitted, and instead the stain is allowed to act from fifteen to thirty minutes.

4. Preparation of Sections.

At the autopsy small pieces of the organs are placed in an abundance of absolute alcohol and allowed to remain there two or three days, the alcohol being changed twice. Usually they can then be cut. For this purpose the firmer parts of the kidneys, liver, muscle, etc., are stuck on corks with liquefied commercial gelatin,[1] and then, with the cork, again immersed in absolute alcohol. After another twenty-four hours the objects may be cut by means of a microtome. In order to obtain sections of more delicate organs, they must be embedded in celloidin or paraffin. Before staining the paraffin is completely removed by several changes of oil of turpentine or xylol, and the preparation carried from xylol to absolute alcohol.

II. Cultivation of Bacteria.

1. Nutrient Media.

(A) Non-albuminous (according to C. Fränkel and Voges).

Sodium chlorid, 5 gm.; neutral commercial sodium phosphate, 2 gm.; ammonium lactate, 6 gm.; asparagin, 4 gm., are dissolved in 1 liter of distilled water. We may add 10% gelatin or 1% agar, and thus obtain a non-saccharine nutrient medium which is suitable for most bacteria. By the addition of milk-sugar a milk-sugar nutrient medium is obtained which is free from dextrose. (Lehmann and Neumann.)

To produce bouillon free from sugar according to Th. Smith's method, beef-extract is inoculated with Bact. coli, allowed to stand twelve hours in the incubator, and sterilized after the addition of peptone and sodium chlorid. Such a non-saccharine bouillon, which is also, after so short a time, free from indol, is better for testing indol production than that prepared in the ordinary manner.

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[1] One part of gelatin is dissolved in two parts of water.

(B) Albuminous.

1. **Peptone Water.**—In a liter of water 10.0 gm. of dry peptone and 5.0 gm. of sodium chlorid are dissolved and it is then sterilized.

2. **Milk.**—Fresh, preferably freshly centrifugated, milk is placed in test-tubes and sterilized in the steam chamber for one-half hour on two successive days. Milk which contains the spores of the subtilis group (see p. 53) can often not be sterilized in this way.

3. **Litmus Whey** (Petruschky).—The casein is cautiously precipitated from milk by producing a very feeble acid reaction with dilute hydrochloric acid. The filtrate is boiled, filtered and neutralized, and mixed with some litmus. The preparation is not very easy. It can be bought.

4. **Hay Decoction.**—About 10 gm. of dry hay are boiled in a liter of water. The filtrate is filled into tubes and sterilized for two hours on three successive days, the tubes being kept overnight in the incubator, in order to destroy the very resistant spores.

5. **Beer wort** (not neutralized) is allowed to settle for some time, preferably several weeks, after being sterilized, and then it is poured off clear into test-tubes and again sterilized.

6. **Nutrient Bouillon.**—(*a*) *From meat:* 500 gm. of lean beef are boiled in 1000 c.c. of water in an enamelled pot over a flame for one-half hour and filtered. The filtrate (meat infusion) is brought up to 1000 c.c., and to this is added 10 gm. peptone and 5 gm. sodium chlorid. This is placed in the steam chamber until dissolved, and the whole then carefully neutralized with normal sodium hydroxid (phenolphthalein being used as indicator[1]) (see p. 36). It is then filtered, filled into tubes, and sterilized.

(*b*) *From extract of beef:* 10 gm. of beef-extract are dissolved in 1000 c.c. of water, and 5 gm. of sodium chlorid and 10 gm. of peptone added. The solution is then neutralized and well sterilized several times.

7. **Potato Water for Tubercle Bacilli.**—Five hundred gm. of peeled potatoes are rubbed upon a grater and allowed to stand over night in a refrigerator in 500 c.c. of water. The fluid is then decanted and brought up to 1000 c.c., boiled for an hour in the water-bath, filtered, 4% glycerin added, filled in tubes, and sterilized.

8. **Gelatin Nutrient Media.**—(*a*) *Meat infusion-peptone gelatin* (ordinary "gelatin" or "nutrient gelatin" of laboratories): To 1000 c.c. of meat infusion (see nutrient bouillon) are added 100 gm. gelatin,

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[1] For example, 10 c.c. of bouillon required 2.2 c.c. of $\frac{1}{10}$ normal sodium hydroxid solution for saturation; 1000 c.c. of bouillon requires 220 c.c. of the same, or 22 c.c. of normal solution. We usually add only 20 to 21 c.c.,—*i. e.*, a little less,—so as to be sure of having no free sodium hydroxid in the nutrient medium.

10 gm. peptone, 5 gm. sodium chlorid. It is warmed in the steam chamber until all is melted, then neutralized with normal sodium hydroxid, filtered, and sterilized. After the liquefied gelatin is filled into tubes, it is again sterilized.

(*b*) *Meat infusion gelatin:* The same as *a*, but without the addition of peptone and sodium chlorid.

(*c*) *Beer-wort gelatin:* Is obtained by the addition of 10% gelatin to the wort. It is not neutralized.

(*d*) *Plum decoction gelatin:* 500 gm. of dried plums are boiled in 500 c.c. of water; the fluid is then poured off, and the plums again boiled in 500 c.c. of water. Both fluids are then mixed, filtered, and 10% gelatin added. It is not neutralized.

(*e*) *Herring gelatin:* Two salt herrings, unwashed, are boiled in 1 liter of water, and to the filtrate 10% gelatin is added. It is not neutralized.

(*f*) *Potato-water gelatin:* according to Holz, for Bact. typhi: 500 gm. of potatoes are carefully washed, peeled, finely grated, and squeezed through a linen cloth. The turbid juice may be allowed to settle for twenty-four hours and is then filtered, or, as we always do, it may be filtered at once through animal charcoal, and, after heating, if necessary the filtration is repeated. After heating for an hour in the steam chamber, 10% gelatin is added to the clear fluid, when it is again heated in the steam chamber, filtered, filled into tubes, and sterilized on three successive days. It is not neutralized. (H. K. Lang.)

(*g*) *Potassium iodid potato-water gelatin* (Elsner): 1% potassium iodid is added to prepared gelatin. This is best done by adding the required amount of strong sterilized solution to prepared gelatin just before using it.

9. **Nutrient Agar.**—To 1000 c.c. of meat infusion 10 gm. of fine cut agar are added and the mixture is boiled in a glass flask over an open fire for one hour until the solution is complete; then the evaporated water is replaced and 10 gm. peptone and 5 gm. sodium chlorid are added. After again heating in the steam chamber the fluid is neutralized and filtered by means of the hot-water funnel, filled into tubes, and sterilized.

10. To obtain **grape- or milk-sugar agar,** 2% of either substance is added simultaneously with the peptone and salt. Since meat-infusion agar usually contains traces of grape-sugar, we have for some time prepared a milk-sugar agar which is free from grape-sugar according to the method described under A.

11. **Glycerin-agar.**—To the prepared nutrient agar 5% of glycerin is added, when it is filled into tubes and sterilized.

12. **Sugar-chalk Agar.**—Usually finely pulverized, dry, sterilized carbonate of calcium is added to liquefied sugar-agar in sufficient quantity to render it cloudy and opaque. It is then inoculated with the bacteria and poured into plates.

13. **Potatoes.**—After thorough washing and rinsing the potatoes are peeled, cut into slices 1 cm. thick, and sterilized several times in deep Petri dishes. The peeled potatoes may also be perforated with a large cork borer and the cylinders be divided by an oblique cut into

two parts. The pieces are then placed in test-tubes with a little dry cotton at the bottom (to absorb the water of condensation) and sterilized several times in the steam chamber.

14. **Blood-serum.** — Blood, obtained from slaughtered animals under proper precautions, is allowed to stand in a refrigerator in well-cleaned glass cylinders for twenty-four hours. Then the serum is removed with large sterile pipets. It is then placed in flasks with the addition of 1% of chloroform and allowed to stand several weeks, being occasionally shaken. Before using, the tubes, into which the serum has been filled, are placed in the incubator for a few days to insure complete evaporation of the chloroform. It may be used as a fluid or after being solidified at 65°.

15. **Löffler's serum mixture** for diphtheria bacilli: Three parts of beef- or sheep-serum are mixed with 1 part of veal bouillon which contains 1% grape-sugar, 1% peptone, and 0.5% NaCl.

16. **Ascitic Fluid, Fluid from Ovarian Cysts.**—The fluid obtained by puncture has added to it a little chloroform (30–50 gm. to liter) and is stored in a dark place for several weeks or months, being frequently shaken. If the fluid is clear like water it is drawn into a sterile pipet and filled into test-tubes. The tubes are placed in a water-bath for half an hour at 30°–35° to drive off the chloroform.

Glycerin-ascites-agar is prepared by mixing equal parts of the above fluid and of 2% nutrient agar which has been liquefied, cooled down to 40°, and which contains 5% glycerin. This medium replaces blood-serum in most cases, and upon it we have observed a very good growth of the organisms of gonorrhea, pneumonia, tuberculosis, whooping-cough, and diphtheria, and of streptococci and Bact. duplex (Morax's diplobacillus).

17. **Silicic acid nutrient medium** is very different from the other media. It was first devised by Kühne, and has been modified by various authors. Gelatinous silicic acid, which is merely mixed with certain salts, is an important nutrient medium for some organisms (for example, the nitrate producers) because of the lack of organic nutrient substances. For the rather complicated preparation consult Stutzer and Burri (C. B. L. I, 722).

18. As a substitute, Beijerinck has recommended a **water agar,** prepared by extracting for a long time with distilled water, from which the nutrient substances are removed by decomposition and diffusion and to which the salts may be added (C. B. XIX, 258).

19. **Cerebral Nutrient Medium** (v. Hibler).—Human brain from fresh cadavers is cut up finely with a chopping machine, placed in small flasks, and stirred up with enough distilled water to form a semifluid infusion. The masses are then sterilized. Before using, the nutrient medium is again boiled for half an hour.

20. **Nutrient Medium for Differentiation of Species of Actinomyces.**—The following is recommended by Gasperini:

Wheat flour	150
Water	1000
Magnes. sulph.	0.5
Potass. nitrate	1.0
Grape-sugar	15.0

2. The Employment of the Different Nutrient Media Depends upon the Following Points of View:

I. Fluids (Bouillon, Sugar-bouillon, Milk, Non-albuminous Nutrient Media)

are employed:

1. To produce culture *en masse.*
2. To obtain suspensions of bacteria in which the number can be accurately determined (counting by means of plates).
3. For the study of the formation of pellicles and sediments.
4. For the study of the metabolic products (compare p. 58 and what follows).

II. Solid Nutrient Media.

1. Gelatinous Nutrient Media.—The gelatinous, transparent nutrient media (agar and gelatin) are most extensively employed for the following reasons:

(*a*) They may be employed as fluid and solid nutrient media: as fluids, allowing a separation of the bacteria; and as solid substances, a fixing of the isolated germs and their separate growth into colonies.

(*b*) On account of their transparency they allow a macroscopic as well as microscopic observation of the cultures; they allow a thorough differential diagnosis of varieties and an early recognition of any contaminations.

They are especially used: (*a*) For plate cultures—*i. e.*, for demonstration, for accurate separation and counting of the individuals and varieties.

(*b*) For obtaining characteristic, macroscopic cultures which serve in differential diagnosis.

(*c*) For permanent cultures, or collections of living bacteria.

The special advantages of agar and gelatin are:

(*a*) *Gelatin.* — *Advantages:* Easily prepared, readily made into plates (at 25°); the property of being liquefied by many bacteria is of great diagnostic value. *Disadvantages:* Since it melts at 25° it cannot be used in hot weather nor at incubator temperature.

(*b*) *Agar.*—*Advantages:* It may be used at incubator temperature (*i. e.*, for the rapid growth of bacteria—spores—and especially thermophilic bacteria). *Disadvantages:* Difficulty of preparation, more difficult to make plates from (the agar, melted at 80°, must be cooled to 40° before being inoculated). Colonies are often not characteristic.

2. Blood-serum, glycerin-agar, and glycerin-ascites-agar.—Employed especially for growing pathogenic varieties, which thrive poorly or not at all on other nutrient media. It is only possible to make plate cultures with glycerin-agar and mixtures of agar and serum.

3. Potato.—(*a*) To obtain macroscopically characteristic cultures of great durability and for differential diagnosis.

(*b*) Sometimes for spore-formation.

3. A Few Words Regarding the Technic of Ordinary Cultures.

The platinum needle must be heated red-hot each time before it is used and before putting it down.

(*a*) **Fluid culture media** are inoculated with a loopful of pure culture.

(*b*) **Gelatin and agar stab cultures** are made with a straight needle, only a single stab being made in each tube, but it should extend almost to the bottom of the tube.

(*c*) **Agar and gelatin streak cultures** and **potato cultures** are inoculated by a gentle superficial stroke over the surface with the platinum loop. It is sometimes necessary to rub the culture into the potato.

(*d*) **Gelatin plate cultures:**

1. **To isolate certain bacteria in pure culture:** The gelatin in three tubes is melted, and after it is cooled down to 30°, a loopful of a fluid or a trace of a solid pure culture is introduced into one of them and well mixed. From this first tube one or two loopfuls of gelatin are carried to a second tube, and from this, after mixing, two or three loopfuls are again transferred to the third tube. After anything which may be upon the edge of the tubes has been burned off, the contents of each tube are poured into separate sterile plates, the cover being quickly raised for this purpose, and the plate inclined gently to and fro in order to distribute the gelatin as uniformly as possible. During the transferring from one tube to another it is recommended that they be held inclined, to prevent the falling into them of foreign germs. The plates thus prepared are then placed in a culture chamber with a constant temperature of 22° (or room temperature is used), and after two or three days the individual colonies which have developed are studied macroscopically and with slight (fifty times) magnification. Usually, of the three plates, only two are useful; at least one has been sown too thick or too thin.

2. If one wishes to ascertain **the number of bacteria**, for example, in water, 1 c.c., 0.5, and 0.1 c.c. of the water is placed in three tubes of liquefied gelatin, well mixed, and poured into plates. To ascertain the number of germs, if they are very numerous, the Wolfhügel counting plate is used; if only a few colonies appear, then the plate is inverted, the bottom divided into sextents with ink, and each visible colony marked with a dot. Plates made to determine the number of bacteria in drinking-water must be counted several times (on the second, third, and fifth days). In the case of fluids with very many germs (sour milk, canal-water, etc.), 1 c.c. is first placed in 100 c.c. of sterilized water, and this then treated as above. Solid bodies are first rubbed up in water. In the examination of air a definite quantity is drawn through a tube filled with sterilized sand, the sand then being washed in sterilized water and plates prepared from it.

(*e*) **Agar plate-cultures** are prepared in the same way. The agar must not be too cool when poured into the dish or it will solidify at once, forming an uneven surface. On the contrary, if it is too hot, the bacteria are killed by the temperature. Recently it has been much advised that, in making agar (partly also gelatin) plates, the nutrient medium be first allowed to solidify in the dishes, and then the surface be superficially smeared over with the material to be examined by means of a platinum loop, strips of filter-paper, or a platinum brush. Only characteristic surface colonies are obtained in this way.

(*f*) **Sugar-agar shake cultures:** The contents of a tube are melted in the water-bath and cooled down to about 40°. A loopful of the pure culture is then introduced and thoroughly distributed, and after the medium solidifies the tube is placed in the incubator.

4. Anaerobic Cultures.

We have employed almost exclusively the method of H. Buchner: Absorption of oxygen by pyrogallic acid in the presence of potassium hydroxid.[1]

(*a*) *For Stab Cultures.*—At the bottom of a glass cylinder, which must be a little longer than the test-tube, is placed a heaping teaspoonful of pyrogallic acid and 20 c.c. of 3% potassium hydroxid solution. The inoculated stab culture is then placed in the cylinder, which is closed at one end with a soft rubber stopper or a ground-glass stopper which is sealed with paraffin. According to Kitasato, anaerobes which are less sensitive to oxygen may be cultivated in high stab cultures in sugar-agar without pyrogallic acid. A stab 8 to 10 cm. deep is made in sugar-agar with a small loop and the needle turned upon its long axis before being withdrawn.

(*b*) *For Plate Cultures.*—Instead of the glass cylinder, a wide exsiccator with a ground cover is used. The lower part is filled with sand and the pyrogallic acid mixture, and then the manipulation is as above (Arens).

If it is desirable to obtain the most perfect anaerobiosis, the pyrogallic acid method is combined with either the pumping out of the air with a water-pump or the displacing of the air with hydrogen, so that only a slight trace of oxygen remains to be taken up by the pyrogallic acid. We have employed the latter method many years. The cultures are placed in a roomy exsiccator with sufficient pyrogallic acid and potassium hydroxid, and then, by means of a double perforated rubber cork, hydrogen is allowed to flow through for one-half hour. After closing the opening, we sink the whole apparatus, weighted with lead, in water.

Kabrhel recommends (C. B. XXV, 555), as a control for the absence of oxygen, that a tube be introduced which contains liquefied nutrient gelatin, to which is added, just before use, 0.3% to 1.0% grape-sugar, and which is rendered a transparent blue with a strong alcoholic solution of methylene-blue. Such an uninoculated tube is completely decolorized in twenty-four to thirty-six hours only in a chamber entirely free from oxygen. This indicator will also point out how essential it is to remove covers, corks, etc., in the case of anaerobic cultures.

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[1] Sensitive varieties are said to thrive better in an atmosphere of lrogen.

III. Animal Experiments.

(A) Infection.

1. Subcutaneous Inoculation.—After the skin in some part has been washed with 1 : 1000 corrosive sublimate solution, a shallow incision is made with scissors, and inoculating material is introduced beneath the skin by means of a stout platinum wire with a loop. Mice are usually inoculated above the root of the tail, they being simply held by the tip of the tail and allowed to hang into a glass which is covered in great part by a piece of board. Guinea-pigs and rabbits are inoculated on the side of the thorax.

2. Subcutaneous injection is usually carried out with Koch's rubber-ball injection syringe or with Strohschein's syringe. A fold of skin is picked up upon some part of the body, and the needle introduced in the direction of the fold. If several cubic centimeters are to be injected, it may be simply done as follows : Upon a graduated pipet is fastened a short piece of rubber tubing provided with an injection needle, and the whole sterilized. The pipet is sucked full, and the fluid forced out with the mouth or a rubber bulb.

3. Intraperitoneal injection is made by perforating the abdominal wall at a single thrust with a sterile hollow needle ; then, cautiously advancing the needle, the fluid is injected.

Regarding infection by feeding, inhalation, etc., consult more extensive works on technic.

(B) Observation.

Mice may be kept in sterile glass vessels provided with cotton and closed with wire gauze. Larger animals must be kept in sterilized cages or stalls.

(C) Autopsy and Disposition of the Body.

Autopsies must be made at once after death; at least, the animal must be placed on ice after death. The animal is placed upon a board on its back and nailed or tied by its four legs. The abdomen and chest are thoroughly moistened with sublimate solution and then the abdominal cavity first opened with a sterile knife. The abdominal walls are separated, and from the spleen, liver, and kidneys, some blood (or tissue juice) is obtained with a sterile platinum loop and smeared at once upon previously prepared agar plates. The organs are carefully cut out, avoiding contact with the intestines, and placed in absolute alcohol for further examination. Then the thorax is opened with scissors, and blood removed from the heart and also the lungs. These organs are also placed in alcohol. Before each operation the instruments must be carefully heated to a glow or thoroughly burned. It is better to have numerous sterilized instruments ready. The hands must be perfectly clean.

In interpreting the findings at the autopsy it is to be remembered that often very soon (sometimes during the death agony) micro-organisms migrate into the organs from the intestine. If living bacteria are injected into the abdominal cavity or trachea of cadavers, they can very often be found in the organs after a time (C. B. XXIII, 418).

After the autopsy the body is best burned. If this is not practicable, the body is wrapped in coverings wet with sublimate and buried at least 0.5 meter deep, and quicklime filled in about it.

APPENDIX V.

Brief Guide to the Recognition of Bacteria.

(Illustrated with an example.)

The case is one of eczematous conjunctivitis in which a number of the bacteria occurring in diseased eyes are present. Purulent or serous material removed from the conjunctival sac or edge of the lid with a platinum loop is made use of.

I. Microscopic Examination (Smear upon Slide or Cover-glass).

(a) **Stained with fuchsin,** we see:

1. Cocci, especially diplococci in heaps, usually distinctly "biscuit-shaped," many times within cells (perhaps gonococci).
2. Cocci, single or united in irregular clusters (probably Micro. pyogenes).
3. Short chains, of two or three links, of lance-shaped cocci, some with capsules (probably Streptoc. lanceolat.).
4. Rods, larger or smaller, often very irregular in form, staining in segments, ends rounded or pointed, often of the size of cocci (true diphtheria, pseudodiphtheria, or xerosis bacillus).
5. Rods regular, rather thick, but small (perhaps coli group).
6. Rods, often in pairs, quite large, the ends not rounded (perhaps, although at the time without spores, a bacillus or Bacterium duplex).

(**b**) **Gram's stain:** All the organisms in the preparation are stained except the biscuit-shaped cocci and the small, plump regular bacteria. The loss of color speaks in favor of the cocci being gonococci, and the rods Bact. coli.

(**c**) **Stain for tubercle bacilli** with carbol fuchsin: In the differentiation the preparation is completely decolorized with sulphuric acid. After counter-staining with methylene-blue only organisms which are stained blue are seen. Thus, in our case the tubercle bacillus and those resembling it are excluded.

If, as sometimes occurs, no micro-organisms can be seen in the fuchsin preparation, then a preparation is also stained by Gram's method because the stained cocci and bacilli are more readily seen after the mucus and coagulum have been decolorized. In any case if the examination of the slide is negative, the plate method is always employed.

II. Plate Cultures.

In examining an animal body for micro-organisms the nature of which we do not know, we employ "the best nutrient medium": *i. e.*, serum or ascitic fluid-agar, and, as a substitute, glycerin-agar.[1]

The usual plate method consists in placing the material to be examined in liquefied gelatin or agar in various dilutions and pouring it out into double dishes. This is not especially suitable for the examination of materials which contain relatively few germs. In our case we prefer to pour the nutrient medium into plates, and, after it is solidified, to carefully make several streaks over the surface with a platinum loop which carries the pus or mucus, etc., to be examined. The double dishes are then turned upside down (so the agar will not dry so rapidly) and placed in the incubator.

After forty-eight hours there appear upon the plate:

1. Moist, white, yellow, and orange, roundish, slightly elevated colonies,[2] which, when magnified sixty times, are

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[1] On the contrary, many varieties from soil, water, etc., grow only upon nutrient media poor in nutrient substances, like the ordinary nutrient media (see p. 200).

[2] The colonies here described are always such as lie on the surface of the medium.

finely granular. In stained preparation, magnified a thousand times, they are micrococci (probably Micrococcus pyogenes albus, citreus, and aureus). The examination must be carried further, as indicated on page 163.

If there are only one or two colonies,—especially yellow ones,—one may often recognize it as a contamination of the plate by germs in the air, most often sarcinæ. The edges of the sarcina colonies, when magnified sixty times, are coarsely granular or jagged. When magnified one thousand times, packets of micrococci are seen.

2. Most minute, scarcely perceptible colonies, not elevated, half a millimeter in diameter. When magnified sixty times, extremely delicate, transparent, very delicately punctate. The edges practically smooth (recalling gonococcus, Streptococcus lanceolatus, and Streptococcus pyogenes). In the last, one often observes upon ascites-agar that chains of cocci grow out from the edge of the colony in the form of the finest curling threads! When the stained preparation is examined under a magnification of 1000, the examination is to be continued according to page 163, if cocci; page 134, if streptococci; page 195, if bacilli.

In order to render still more secure the diagnosis founded upon morphologic and biologic peculiarities, several such small colonies are taken up with a platinum loop and introduced beneath the skin of a mouse, or this may be done by employing more abundant infecting material (bouillon culture). If we are dealing with a Streptococcus lanceolatus, we find in the blood and organs characteristic forms of this variety with capsules. Smears from the blood and organs are to be examined for the characteristic organisms (Strept. pyogenes, Strept. lanceolatus), and also new smear inoculations made upon nutrient media.

3. Tiny white to yellowish-white points, rather dense, and just visible with certainty after twenty-four to forty-eight hours. If the plates are kept longer, there is usually only a slight increase in size, up to about 0.5 mm., and then, with very few exceptions, they become no larger. They are, however, always distinguished from those named before by the tougher consistency. When magnified sixty times, the border is ragged, often as if gnawed away, and

splintery (see Plate 59, 1) and of a yellowish color. Magnified 1000 times: Stained in segments, highly polymorphous, short, long, thick, thin, clubbed, pointed, also with the form of cocci (apparently diphtheria or pseudodiphtheria or xerosis bacilli). In the pseudodiphtheria bacillus the border is often coarsely granular, similar to sarcinæ. The further examination is conducted according to page 384.

4. Larger, moist, sometimes slimy, luxuriant colonies, somewhat elevated, whitish to gray, with transmitted light somewhat iridescent. When magnified sixty times, the edge is smooth. Microscopic preparation magnified 1000 times: Small, plump or more slender rods, perhaps also isolated short chains. Not stained by Gram's method. Belongs to the group of non-sporulating bacteria. Perhaps or probably the Bact. coli or a closely related variety. It is to be further studied regarding motility, gas-formation, indol, coagulation of milk, according to page 169, etc. When transferred to gelatin plates, the colon group presents, upon slight magnification, the characteristic, wavy, smooth-edged, transparent colonies with intersecting lines.

5. Macroscopic: Similar to the colonies described under 4, but never slimy; grayish-white, often gray. When magnified sixty times, the border is matted or curly. Microscopic preparations magnified 1000 times show sturdy bacilli, of equal length, the ends not rounded, staining by Gram's method, often united in chains (very probably sporulating organisms of the subtilis, anthrax, and mesentericus group). To be further studied according to page 304.

6. Essentially the same as under 5, but the periphery is exceedingly delicate and transparent. There is neither observed formation of curls nor irregular breaking up of the periphery into a felty structure. The microscopic preparation magnified 1000 times shows bacilli similar to those described under 5, but usually arranged in pairs (probably Bacterium duplex).

It may here be again stated for the beginner that the diagnosis, especially the separation of the bacteria into separate groups, may be much facilitated by paying attention to the **periphery of the colonies.** In the following table are entered the appearances occurring in the most important varieties. Exceptions occur, of course:

NUTRIENT MEDIUM.[1]	VARIETY.	BORDER OF SUPERFICIAL COLONIES MAGNIFIED SIXTY TIMES.
Agar.	**Streptococcus pyogenes.** **Streptococcus lanceolatus.** **Micrococcus gonorrhœæ.** **Bacterium influenzæ.**	Smooth or only extremely finely granular. Exceptions: many Streptococci pyogenes on ascites-agar.
Agar.	**Micrococcus pyogenes** and all luxuriantly growing micrococci.	Finely granular.
Agar.	**Sarcinæ.**	Coarsely granular, often as if eaten away. In many varieties individual packets are distinctly seen at the periphery.
Gelatin.	**Bact. typhi and coli, and related organisms.**	Wavy, smooth. In young stages, fine lines as if cut in, passing from border toward center.
Gelatin.	**Liquefying air and water bacteria.**	Beset with most delicate little hairs.
Agar and gelatin.	**Subtilis group and anaerobic bacilli.**	Periphery broken up into irregular, tangled locks.
Agar, less in gelatin.	**Anthrax and closely related organisms.**	Regular, beautiful formation of curls and locks.
Gelatin.	**Vibrios, especially cholera.**	Scalloped to finely lobulated, in the interior moruloid. Later the periphery is crumbly, until it is finally entirely disintegrated.
Gelatin and agar.	**Diphtheria bacilli and its relatives.**	Similar to sarcinæ, but irregularly cut and fringed.
Glycerin-agar.	**Tubercle bacilli.** **Actinomyces and their relatives.**	Smooth, very wrinkled, cartilaginous, distinguished by strong reflex.

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[1] The nutrient media are cited upon which the variety concerned grow[illegible]eristically.

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